SCIENCE

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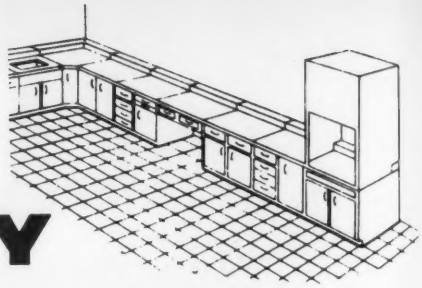

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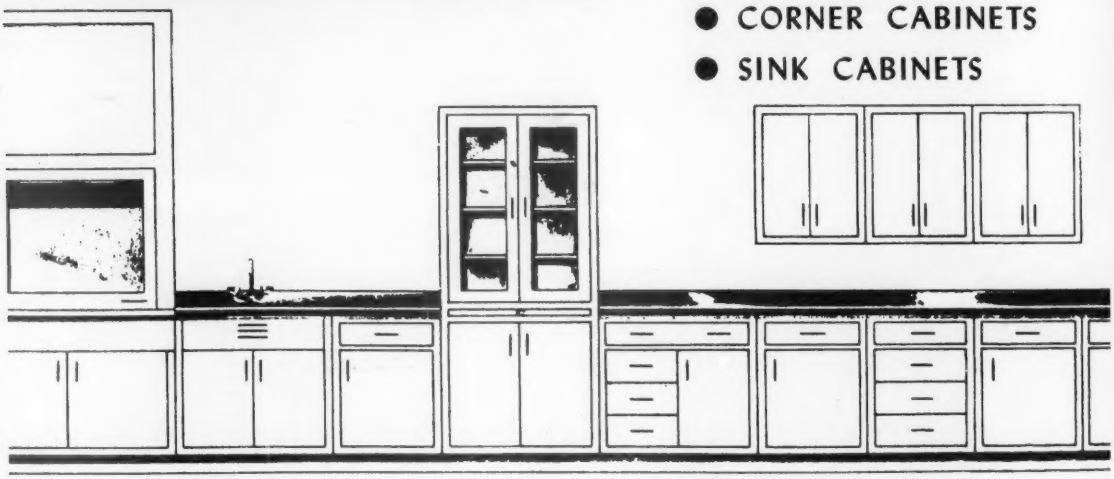

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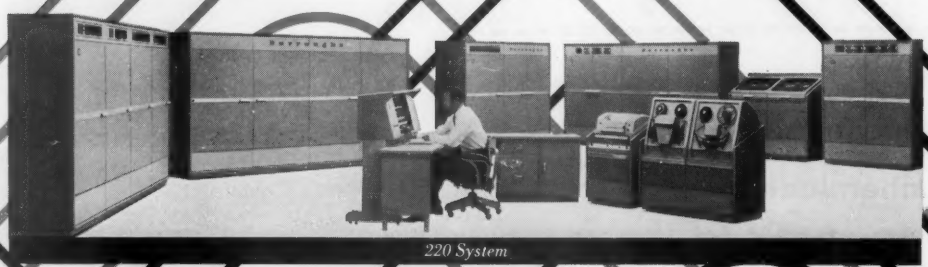

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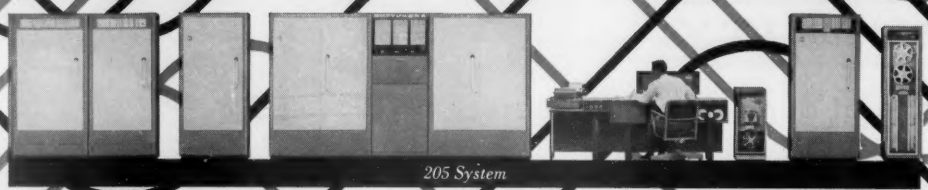

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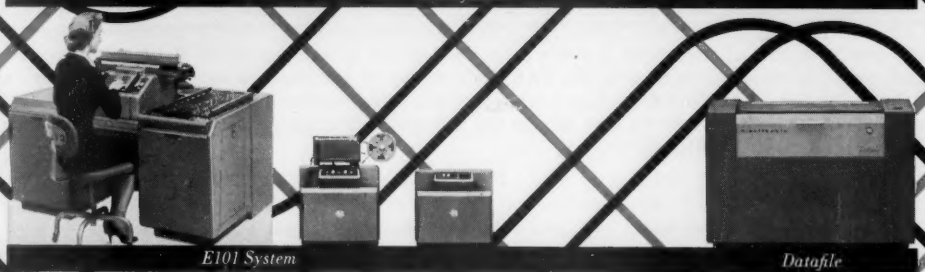

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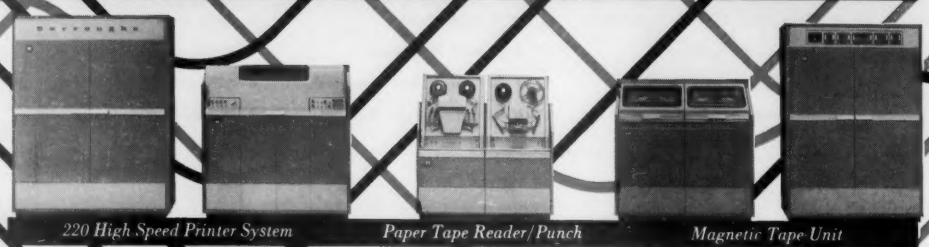

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WE'RE LEARNING WHY TWO EARS ARE BETTER THAN ONE

Which speaker is making the sound? In echoless chamber at Bell Labs, Robert Hanson measures test subject's ability to localize sounds — observes how two ears operate in partnership. This and other tests may point the way to better telephone instruments.

In listening to stereophonic music, how is it that our ears and brain construct a picture of the entire orchestra with but two samples (the sounds from two speakers) to work with?

How is it that our ears and brain are able to pinpoint *one* voice in a roomful of talkers—to listen to it alone and ignore the rest?

What makes *two* ears better than one?

Bell Telephone Laboratories scientists are searching for the answers. For in finding them, better telephone instruments and better ways of transmitting sound will surely result.

Our hearing performs feats that no electronic system can yet duplicate. How? Laboratories scientists believe the secret lies in the way our two ears function in partnership and in the way our neural network connects them with our brain. *The problem:* to discover what functions the network performs and to see whether electronic duplication might enhance understanding.

The work is under way. Electronic circuits that simulate the operation of nerve cells have already been created—and conceptual models of the neural network are being constructed.

Alexander Graham Bell's interest in deafness and hearing led to the invention of the telephone. Bell Laboratories' current explorations in binaural sound may well lead to important new advances in the transmission of speech and music.

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World center of communications research and development

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SCIENCE

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Preface to a Prolegomenon

When the Geneva talks on suspending nuclear tests resume on 8 June, there is a possibility that the impasse of on-site inspection may be avoided. The Western powers have argued for such inspection because an earthquake that instruments are unable to identify as of natural origin might be the result of an underground nuclear explosion. The Soviet Union has argued that inspection should be subject to veto by the participants because it presents opportunities for espionage. Prime Minister Macmillan proposed a way out of the impasse which was seconded by Premier Khrushchev and which was introduced officially into the Geneva test talks by the Soviet delegation before the current recess. The proposal was to limit the number of veto-free inspections permitted each year.

The American delegation has been sufficiently interested in the proposal to join the British delegation in asking the Soviet delegation to elaborate on a number of points, among which are: how many inspections should be permitted each year? and what should be the scientific criteria for determining when an earthquake is a candidate for inspection? Although these questions concern provisions to be written into a test treaty, the answers would seem to require a scientific opinion on the technical implications of such matters as the number of natural earthquakes occurring annually in various parts of the world. But before such an opinion can be rendered, the Western powers and the Soviet Union must agree to assemble the necessary experts.

Moscow has at times been willing and at times unwilling to allow Soviet scientists to meet with Western scientists. Soviet scientists participated in technical talks last summer, and these talks led to the political talks now in recess. Early this year, however, Moscow was not willing to call in Soviet scientists to examine the new data concerning the differentiation between natural and artificial earthquakes that the United States developed in the course of its project Hardtack. The reason for the refusal probably was that such a study would have indicated the need for a more extensive inspection system than the one based on information developed at the summer talks. Moscow recently expressed interest in exploring further the means for detecting high-altitude explosions, but this gain does not apply to the present problem since such explosions offer no site to inspect.

Scientific talks may be a necessary prolegomenon to political talks, but the agreement to conduct scientific talks requires an initial political accord. The problem of determining a suitable limit to the number of on-site inspections is important not only in its own right but as an illustration of the need to include in the test treaty a mechanism for convening future scientific talks. Since it is likely that advances in science requiring modification in the system will occur, East and West must agree to recognize such advances, evaluate them, and revise the system accordingly. The inclusion in the treaty of a clause allowing for the revision of other clauses is a condition that we trust the Western powers will insist on and one we hope the Soviet Union will accept.—J.T.

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CURRENT PROBLEMS IN RESEARCH

Antibiotics

The exploitation of microbial antagonisms is having a challenging impact on medicine and society.

Paul R. Burkholder

Antibiotics, in the broadest sense, are substances produced by living organisms, which, in small amounts, can inhibit the life processes of other organisms. The word *antibiotic, in sensu strictu,* is used for a chemical substance, of microbial origin, that has the capacity to inhibit the growth of bacteria and other microorganisms and even to destroy them. Antibiotic substances are of general interest in chemistry and biology, but their greatest impact on science and society has come about through practical applications in medicine and agriculture.

New Concepts of Antibiosis and Chemotherapy

It is well known that antibiotics were not the first effective, specific drugs used in the medical armamentarium for conquest of human disease. Very beneficial remedies of plant origin were introduced into Europe for use against malaria and amebic dysentery as far back as the 17th century. There was at that time no underlying rationale or basic conceptual scheme of chemotherapy. Cinchona bark and ipecacuanha rested on their own merits as simple remedies, produced in nature and discovered through early empiricism. Knowledge about the active drugs quinine and emetine seems not to have stimulated much scientific endeavor beyond continuing investigations of remedies found in the primitive pharmacopoeia.

The rationale of modern chemotherapy is based upon knowledge of the etiological agents of infectious diseases and knowledge of the properties of selected chemical compounds which differentially inhibit the growth of pathogens without doing undue harm to the host. It was Paul Ehrlich's concept that chemical constitution determines biological effect, which led to the development of Salvarsan against syphilis and, later, of the sulfonamides against streptococcal diseases and of pyrimethamine against malaria. The objective of Ehrlich's kind of chemotherapy is to discover drugs which act selectively upon essential constituents of pathogenic microbes —charmed bullets logistically designed for specific target sites inside the invading organisms.

When the conceptual scheme is clear, it is relatively unimportant whether 606 or 914 experiments are required to attain the desired goal. In rational chemotherapy, the elegant methods of organic chemistry can lead to successful synthesis of antimetabolite drugs and other types of nice remedies. Since the search is always more satisfying when desirable goals can be defined, we cannot afford to overlook the many indications provided by nature's antibiotic compounds, which may serve as guideposts toward a more rational chemotherapy. It appears that in our present state of relative ignorance it is no more and no less rational to test the inhibitory powers of synthetic analogs of growth factors against pathogenic bacteria than to assay the spectrum of microbial inhibition for a novel antibiotic "beer." One approach may confirm the educated guess about a possible mechanism of inhibition; the other may lead to discovery of some metabolic pathway that has been blocked; both approaches have provided desirable therapeutic agents—for example, *Salvarsan* and *penicillin.*

The natural phenomenon of antibiosis is not a new discovery of our present generation. A century ago, Louis Pasteur (1857) observed that onion juice inhibits growth of lactic acid bacteria without affecting certain other kinds of microorganisms. A little later, Pasteur (1877), Babes (1885), Garré (1887), and others noted how certain common bacteria could stop the growth of the anthrax bacillus and other kinds of microbes. It was even suggested by these early microbiologists that some day antagonistic properties of microorganisms might be used to combat infectious agents of disease. The scattered data on microbial antagonism in the late 19th century were not integrated into any formal scientific doctrine. Had it been otherwise, an earlier crystallization of the antibiotic concept might have directed research sooner toward desired goals—for example that of finding how better to prevent and alleviate human suffering by means of specific medication.

The word *antibiosis* was first used by Vuillemin in 1889, to describe the phenomenon where one organism is in opposition to the life of another. Actually, several antibiotic preparations, such as pyocyanin, pyocyanase, prodigiosin, and mycophenolic acid, were prepared before 1900, but unfortunately none of these could be developed for use in chemotherapy because of their ineffectiveness and toxicity. Antagonistic relations among microorganisms were not completely forgotten after 1900. According to a monograph on microbial association published by Papacostas and Gaté in 1921, an inhibition resulting from the association of two microbes in vitro is called *antibiosis* (*anti—against, bios—life*). Progress in this field seems to have

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rested, in the first quarter of the 20th century, with recognition of the important but generally overlooked fact that microbial antagonism does indeed exist.

The Antibiotic Era

The next great and independent advance came in 1929, when Alexander Fleming observed a *Penicillium* mold destroying the bacterium *Staphylococcus aureus* in an old discarded petri dish. His imaginative genius moved Fleming to go beyond the usual explanation—that of stale products—to coin the term *penicillin,* the name for a new specific substance capable of killing bacteria. Fleming then proceeded with studies on the powerful action of penicillin against many common pathogenic bacteria and showed that it was not toxic to animal and human tissues. The development of penicillin was not deemed practicable, however, until 10 years later, when the Oxford group—Florey, Chain, Heatley, and others—began reinvestigations into the medical merits of Fleming's almost forgotten discovery. Penicillin soon became the first and greatest of antibiotic drugs, largely because of engineering accomplishments in the fermentation industry.

Another discovery in the field of antibiotics came at just the right moment. Although penicillin was being developed for chemotherapy, many persons came to believe that it was unique, and that probably there would be no more big antibiotics. Then, in 1939, René Dubos demonstrated two new crystalline antibiotics, gramicidin and tyrocidin, from a bacterium called *Bacillus brevis.* Publication of this research had great impact upon the thinking of bacteriologists and the strategy of investigators. Now it seemed clear that there might be many more antibiotic substances in nature.

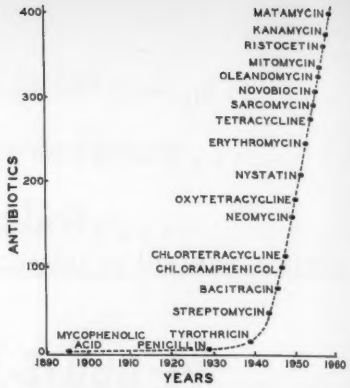
Search for the new substances went forward in many laboratories. Among several antibiotics discovered in Waksman's laboratory, streptomycin, produced by an actinomycete, *Streptomyces griseus,* soon came to be recognized as a powerful therapeutic drug against the microbe that causes tuberculosis. Two important results of the discovery of streptomycin may be noted. First, the battle against tuberculosis began to be waged successfully, and second, some of the accumulated profits from gigantic drug sales made possible the organization of an unusual Institute of Microbiology at Rutgers University, where students are trained and research goes forward in microbiology.

Knowledge in various fields of human endeavor, in that of antibiotics or any other, seems to grow like living populations of organisms. The number of publications on antibiotics has already grown into tens of thousands. Beginning with mycophenolic acid, the first mold antibiotic prepared in crystalline form in 1896, the number of "discovered" antibiotic substances has grown to many hundreds. Along the antibiotic growth curve a few noteworthy compounds have appeared. Some of these are very important in medicine, because of their desirable properties in relation to great human needs. To be very useful in medicine an antibiotic must be more injurious to pathogenic organisms than to the human body. The drug should be comparatively stable, easily absorbed, decisively active against living agents of disease, and without harmful side effects, and when its work is done it should disappear from the patient's body. Only a few antibiotics have measured up to these rigorous requirements.

Following development of the antibiotic concept and successful production of several very important narrow-spectrum antibiotic drugs, such as penicillin and streptomycin, the next great advance came with the advent of broad-spectrum antibiotics. The broad-spectrum antibiotics are capable of inhibiting growth of a broad range of microbes, including Gram-positive and Gram-negative bacteria, rickettsiae, large viruses, and some other organisms. Examples are chloramphenicol and the group of tetracyclines, including oxytetracycline and chlortetracycline. Having a relatively simple structure, chloramphenicol was the first antibiotic to be synthesized on a commercial scale. Because of the effectiveness of chloramphenicol against enteric infections, typhus, and other diseases, its relative freedom from undesirable side reactions, and the comparatively low rate of associated microbial adaptations to resistance, this drug has found an important place in chemotherapy. It is sold under the trade name Chloromycetin. The chemical structures of chlortetracycline (trade name, Aureomycin) and oxytetracycline (trade name, Terramycin) and their relation to the basic tetracycline are completely known. The tetracycline antibiotics are widely used against many bacterial infections and diseases caused by large viruses. Some other antibiotics have also been proved useful in the treatment of animal and human diseases, as is mentioned later in this article.

Numerous attempts are now being made to expand the inhibitory spectrum of antibiotics to include destruction of neoplastic cells. Various methods have been developed recently to make possible the screening of antibiotics against cancer grown in rodents, in embryonated eggs, and in tissue culture. With these techniques, encouraging results are being attained. Tumor-retarding agents have been demonstrated in the mold *Aspergillus fumigatus.* Azaserine is a unique compound showing activity against sar-

Petri plate of bacteria contaminated with a large colony of green mold. As he looked at this plate in 1929, Sir Alexander Fleming envisioned a special antibacterial substance, penicillin, produced by the mold *Penicillium notatum* (P) and inhibiting growth of nearby colonies of *Staphylococcus aureus* (S). [From Florey *et al., Antibiotics* (Oxford University Press, 1949).]



Observation of curious microbial antagonisms in the 19th century led to a virtual era of antibiotics in the 20th century. The "growth curve" shown here represents increase in the number of antibiotic compounds reported in the literature plotted against time, in years. A few named substances are listed as examples of the hundreds of known antibiotics.

coma 180 in mice and against some kinds of microorganisms. Sarkomycin inhibits growth of ascites tumor cells in rabbits. Alazopeptin and puromycin are new antitumor substances. Actinomycin inhibits growth of tumors in rodents and arrests certain types of spontaneous tumors in human beings. Sulfocidin and 6-diazo-5-oxo-L-norleucine (DON) depress the growth of experimental tumors in mice. Some species of mushroom-like fungi are known to produce antitumor substances.

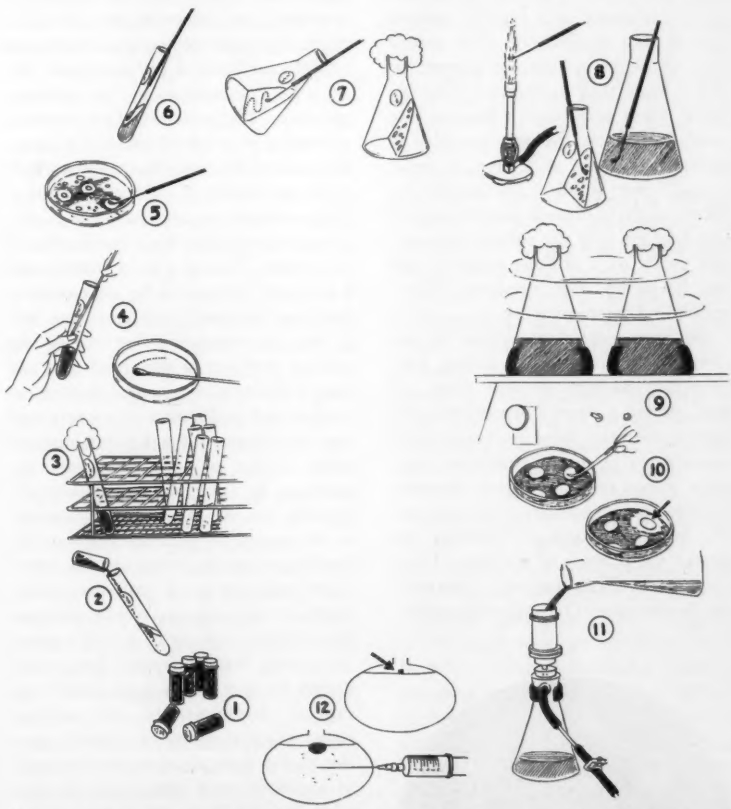
From recognition, in the 19th century, of curious microbial antagonisms has evolved the "antibiotic era" of the mid-20th century. The broadening spectrum of antibiotic action has now been extended to include inhibition of various bacteria, molds, yeasts, algae, protozoa, rickettsiae, large viruses, phages, and finally neoplasia. Perhaps new antiviral antibiotics may provide an approach to the unknown realm of virus-tumor chemotherapy. It is hoped that we may be standing on the threshold of a new era of cancer chemotherapy, and of ultimate prevention or cure of all infectious diseases.

Sources of Antibiotics

Among the numerous kinds of antibiotics which have been discovered, chiefly over a span of the past two decades, many diverse patterns of chemical structure occur. Some species of microbes produce several different kinds of antibiotic compounds, and it is also true that a given compound may be synthesized by more than one species of mold or actinomycete. The rapid growth of the list of new compounds comes about as a result of intensive efforts to find drugs that may be useful against special pathogens and against disease agents that have become resistant to the older antibiotics, making them no longer effective. The majority of the new compounds seem to come from fresh isolates of *Streptomyces,* studied principally in industrial laboratories, in universities, and in governmental institutions in Japan, England, Germany, the Soviet Union, and the United States. Common sources of antibiotics are found among the actinomycetes, molds, and bacteria that live abundantly in soils, composts, and other places. Larger organisms, such as mushrooms and lichens, also produce antibiotic substances which are active against diverse living systems, ranging from bacteria to human cancer. In many flowering plants and in some coniferous trees antimicrobial compounds, of unusual nature or with chemical structures not unlike those of some of the mold antibiotics, are known to occur. A few kinds of algae elaborate antibacterial substances. Some kinds of corals produce compounds which strongly inhibit the acid-fast group of bacteria, to which the tuberculosis germ belongs.

Perhaps it would be stretching the definition of antibiotic substances too far to include the defensive secretions of cockroaches, carabid beetles, and millipedes, although some of these substances bear close structural similarity to certain antibiotic quinones synthesized by some kinds of molds. In the widest usage of the term *antibiotic,* some persons might want to include also Indian arrow poisons, snake venoms, the paralyzing substances of sea cucumbers, the stinging material of the Portuguese man-of-war, or even dinoflagellate toxins which can cause widespread poisoning of fish life in the ocean. The scope of antibiotics in this discussion is restricted chiefly to the various substances produced by certain microbes and active against other microbes.

One of the current problems in antibiotic research is that of developing new ways to find novel antibiotic compounds. The study of specialized source materials from unusual habitats is one approach. Another involves highly specialized enrichments of soil or other material, made for the purpose of encouraging the growth of a minor constituent of the varied microbial population. A



Schematic diagram that shows the 12 steps in the isolation and testing of potential antibiotic cultures obtained from soil. (1) Soil samples are selected. (2) Soil is added to water in test tube. (3) Soil and microbes are now suspended by shaking. (4) A small portion of the suspension is streaked on the surface of nutrient agar in a petri dish. (5) Colonies of microbes grown after 2 days are selected with a bacteriologist's needle. (6) These are transferred to an agar slant. (7) The culture is transplanted into a flask in order to allow further growth. (8) The inoculum is seeded into a flask of nutrient broth. (9) The broth culture undergoes fermentation on a shaker. (10) Potency of the fermented "beer" can be tested with paper pads wet with "beer" and placed on agar plates seeded with living indicator bacteria. (11) An interesting "beer" is filtered to remove microbes. (12) Sterile "beer" is then injected into embryonated chicken eggs bearing implanted human tumors. Inhibition of bacterial growth in step 10, or arrested growth of tumor tissue in step 12, may suggest antibiotic activity of sufficient significance to warrant further studies on selected cultures.

new approach would be the production of mutants which might be blocked in metabolic pathways to yield unusual products.

Quest for Antibiotics

In the search for organisms which produce antibiotic substances, various techniques have been developed, in many laboratories, which are applicable to the survey of large numbers of cultures derived from nature. Small samples of soil, compost, mud, or organic debris are collected from likely places. A portion of the sample is suspended in water and shaken for the purpose of dispersing cells or spores of the numerous microorganisms. Then a small amount of the suspension is spread in a suitable nutrient agar in glass petri dishes. After several days, numerous colonies of diverse microbes make their appearance. The different kinds of molds or bacteria are carefully transplanted into test tubes of sterile media, one at a time, in order to obtain pure cultures. The possible antibiotic activity of each culture may be tested by growing the culture in a suitable broth on a shaking machine and then assaying the resultant "beer" against indicator microbes.

One very convenient method of performing a test consists of seeding indicator bacteria onto an agar plate and then placing a paper impregnated with beer on the agar. After incubation overnight, zones of growth inhibition may occur around some of the pads wherever the beers contain antibacterial substances. The larger the zones of inhibition, the greater the potency of the beer. There are other methods too; each laboratory has its own special techniques for isolating and surveying organisms. New methods would doubtless lead to the discovery of new microorganisms and then of new antibiotics. For testing larger organisms, such as lichens, mushrooms, flowering plants, and corals, extracts of the materials may be prepared and assayed in the same way as when fermented beers of molds or bacteria are used.

The diameters of zones of inhibition around paper pads or cups that contain antibiotics and that are placed on a plate seeded with bacteria are proportional to the concentrations of antibiotic used.

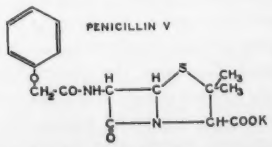
The investigator who has accumulated large numbers of antagonistic microbes must determine which of his cultures produce new and valuable antibiotics. Several means may be useful in discovering whether the activity is caused by one of the many known substances or by some new compound. The colonial morphology, microscopic structure, and physiological characteristics of the organism are studied with a view to determining the nature of the antibiotic-producing organism. Varying sensitivities of different kinds of test organisms permit the construction of an antibiotic spectrum. The pattern of this spectrum, consisting of a list of values for microbial sensitivity, sometimes throws light upon the identity of a given preparation. Drug-resistant strains also serve as precise indicators for the identification of unknowns. Thus, if a penicillin-resistant bacterium is found to be susceptible to the beer of a mold obtained from soil, it may be concluded that among the various products of this mold is a substance different from penicillin. Concentration and purification of a compound may be achieved through differential solubility by the use of countercurrent apparatus, by precipitation techniques, through the use of absorption columns, or by means of chromatography. The mobility of active compounds in paper chromatograms is an extremely useful indicator for purposes of identification, the relative positions of invisible active spots being readily detected by auxanographs in agar plates seeded with susceptible organisms. In this method, paper chromatograms are placed upon the seeded agar plates to allow transfer of antibiotic spots from paper to agar, with the resultant appearance, eventually, of growth-inhibition zones corresponding to the original location of the antibiotic substances on the paper.

Once it has been found that an organism produces an interesting and novel compound, the physical, chemical, and pharmacological properties of the compound are determined, and its probable value as a chemotherapeutic agent is studied. The big hurdle for every antibiotic candidate is the test for toxicity to experimental animals. Many promising drugs turn out to be rat poison and never reach the stage of clinical trial. The therapeutic index must be satisfactory before large-scale production is warranted. Out of the many initial candidates, only a few drugs ever reach the practicing physician and the impatient and willing consumer of antibiotics.

Significance of Antibiotics in Nature

The question has often been asked, "Are antibiotics formed in nature or are they artifacts of the laboratory?" It is well known that many fungi and actinomycetes produce antibiotics in sterilized soil supplemented with organic matter. A few organisms have been shown to produce antibiotics when they are grown in sterile soils without added organic matter. Formation of such substances as gliotoxin, chloramphenicol, and actinomycin occurs only in trace amounts under conditions of low nutrient level. In general, it has proved difficult to show that soil microbes produce active compounds in soils containing the normal mixed flora and fauna, unless there has been excessive addition of organic matter. The evidence suggests that antibiotics are detectable in microhabitats of the soil where nutritive conditions are suitable for production of these substances in excess of amounts destroyed by absorption on clay colloids and through breakdown by chemical and biological action.

Several kinds of molds are known to form antibiotic substances such as patulin, alternaric acid, and fusaric acid, found in rotting apples and infected tomato plants. About half of the many lichens which have been examined produce antimicrobial substances in nature. It seems probable, also, that algal antibiotics are not unusual products in the



Many kinds of penicillin are now produced by biosynthesis, selected molds being grown in fermentation broths containing special substances. The structure of penicillin V, shown here, includes a methoxy benzyl moiety joined to the fundamental penicillin portion, consisting of B-lactam and thiazolidine rings, resembling fused structures of the amino acids and cysteine.

natural planktonic environment of lakes and seas. Certain kinds of bracket fungi and toadstools regularly contain extractable antibiotics. Gorgonian corals represent a recently uncovered source of antibacterial substances, but it is not certain whether the animal polyps or their associated algae are the producers. Antimicrobial substances are fairly common among higher plants—for example, in mountain ash, in onions, and in some conifers. The natural functions of antibiotics as products of organic evolution need to be studied much more thoroughly. Formation of antibiotics in nature seems to be compatible with the view that these substances have survival value for the organisms which possess the ability to produce them, as well as for the human beings who have the skill to employ nature's products advantageously in the nutrition of domesticated animals, for crop protection, in food preservation, and for chemotherapy of infectious diseases.

Chemical Patterns of Antibiotics

Perhaps the most interesting aspect of antibiotics from the standpoint of science lies in the comparative biochemistry of their molecular forms and functions. Only a few examples can be mentioned here to illustrate diverse types of organic structures and some of their relationships. It is hoped that investigators may be encouraged to study the metabolic pathways which lead to biosynthesis of antibiotics. Some of the simplest antibiotics bear a close resemblance to the structure of glucose, from which they may be derived in the peculiar metabolism of the antibiotic-producing organisms. Examples are kojic acid, from a species of *Aspergillus* mold; claviformin, from *Penicillium claviforme*; and parasorbic acid, which can be extracted from fruits of the mountain ash, *Sorbus aucuparia*. Antimicrobial substances related structurally to the sweet-clover compound coumarin, are dicoumarol and datiscetin. Related to vitamin C are the antibiotics penicillic acid and lichesterinic acid. Odd 7-carbon-ring tropolones are represented by the fungus compounds puberulonic acid and puberulic acid and by thujaplicin, contained in the cedar tree *Thuja plicata*. If one asks how it can be that molds and trees produce similar antibiotics, any simple answer would have to be based upon their inheritance of genes and enzymes through a common ancestry.

Numerous examples of antibiotics are found among the quinones—antibiotics such as fumigatin, spinulosin, and phoenicin, all of which are related to parabenzoquinone. Among the somewhat more complex structures, showing similarity to α-naphthaquinone, may be mentioned juglone, plumbagin, phthiocol, and javanicin. A whole new group of quinocycline antibiotics resemble substituted anthroquinones. The general structure of aspergillic acid is repeated among the products of other microorganisms—for example, pyocyanin, hemipyocyanin, iodinin, and chlororaphin.

Among the numerous antimicrobial substances which occur in lichens and fungi, usnic acid is a basic type, with related substances such as didymic acid, strepsilin, and griseofulvin. Somewhat more complicated are the derived depsides, perlatoric and olivetoric acids, and the depsidones, sekikaic acid and diploicin. Some of these lichen compounds are now being used for medical purposes in Finland.

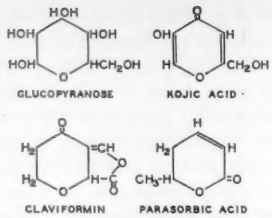
The important tetracycline family of antibiotics is illustrated by oxytetracycline and chlortetracycline. The new antituberculosis drug kanamycin contains two amino glucopyranose moieties coupled to diamino cyclohexane. Steroid antibiotics are known among the metabolic products of bacteria and fungi, such as, for example, polyporenic acids, helvolic acid, and cephalosporin P. It appears likely that these steroids are synthesized from acetate through squalene. The precise structural features which confer on these steroids inhibitory properties against *Staphylococcus* and some other bacteria are not well understood.

A new group of important antibiotics are the macrolides, which have in common a large lactone ring. Included among the macrolides are erythromycin, novobiocin, methymycin, carbomycin, and probably also oleandomycin, pikromycin, and narbomycin. The type compound erythromycin is composed of two sugars, desosamine and cladinose, connected by glycosidic linkages to the 14-carbon lactone erythronolide. This drug is valuable in treating refractory infections caused by various kinds of bacteria. Finally, an important series of antibiotic compounds related to amino acids and peptide groups of these acids should be mentioned. The penicillin group, including penicillin G, V, and so on, as well as the peptide compounds cephalosporin N, micrococcin, and bacitracin, have in common special sulfur- and nitrogen-containing rings. Numerous kinds of penicillin can be biosynthesized by molds in the presence of precursors added to the growth medium. Apparently many kinds of microbes contain enzymes which are able to effect synthesis of compounds containing the thiazoline ring. Not all such moieties possess antimicrobial properties; the thiazole of vitamin B_1 is an important part of the growth-promoting factor required by numerous fungi and bacteria.

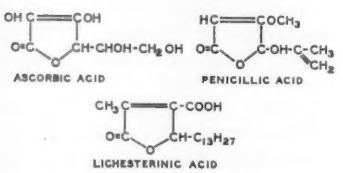
Some of the streptothricin-like antibiotics contain β-lysine. Chloramphenicol may be portrayed as a modified β-phenylalanine. Cycloserine and azaserine are structurally related to serine. 6-Diazo-5-oxo-L-norleucine is a derivative of norleucine. Puromycin contains phenylalanine.

A very large group of polypeptide antibiotics deserves special mention. Numerous kinds of actinomycins have been demonstrated in the fermentation broth of certain kinds of soil actinomycetes. Seven of these compounds have been crystallized and found to contain six amino acids, among which D-valine and D-isoleucine appear to be important in relation to inhibitory properties of the polypeptides. Among other peptide antibiotics containing D-amino acids are tyrocidin, gramicidin, the polymyxins, cephalosporin, and bacitracin. Though many substances of this kind come from actinomycetes and bacteria, some are known to occur in larger fungi—for example, lycomarasmine in *Fusarium* and phalloidine and amanitin in the poisonous toadstool *Amanita phalloides*.

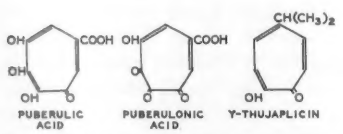
Some of the newer antibiotics possess special properties which make them valuable for therapeutic treatment of specific infections. Thus, nystatin is used in the control of bronchial and intestinal infections caused by *Candida* (yeast), which tends to cause secondary infections in patients following a course of broad-spectrum antibiotics. Fumagillin is particularly valuable for its effective amebicidal action. Streptothricin-like antibiotics include streptocin, roseomycin, neomycin, and xanthomycin. These are not peptide antibiotics, though β-lysine has been identified in some of them. The neomycin complex of substances can be separated by chromatography into a series of different fractions, each with special properties. A group of yellow sulfur-containing compounds include thiolutin, thioaurin, and aureothricin, all of similar structure. Numerous polyenes, isolated from soil actinomycetes, show antifungal activity. The classical polyacetylene structure is illus-



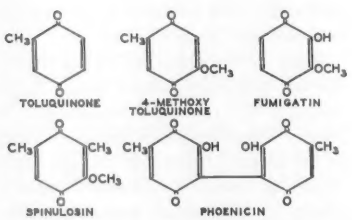
Three antibiotic compounds, kojic acid, claviformin, and parasorbic acid, related in structure to glucopyranose. Kanamycin also contains two amino-glucopyranose moieties.



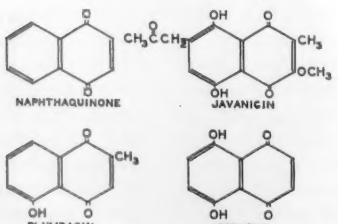
Lichesterinic and penicillic acid resemble ascorbic acid (vitamin C).



Seven-carbon ring tropalones are represented by mold antibiotics, puberulic and puberulonic acids, and thujaplicin, produced by the cedar tree *Thuja plicata*.



Numerous toluquinones are produced by fungi.



Compounds related to α-naphthoquinone are javanicin, of microbial origin; plumbagin, from Indian shrubs of the genus *Plumbago*; and juglone, found in black walnut fruits.

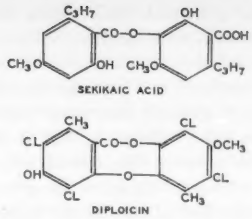
trated by the antibiotic mycomycin. Many other new antibiotics, such as ristocetin, hygromycin, actathiazic acid, and cycloserine, inhibit growth of a variety of different organisms. It is beyond the scope of this discussion even to list all of them by name.

Modes of Action

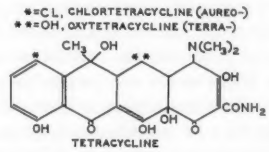
Different kinds of antibiotics work against susceptible microbes in different ways, depending upon special biochemical properties of the drugs and physiological systems of the "bugs." Much research has been done on the ways in which penicillin inhibits growth of Gram-positive bacteria and causes morphological changes of spherical cocci to rod-shaped organisms, or of rods to bizarre filamentous forms. Recent evidence points to blocking of uridine nucleotide incorporation into the cell-wall structure of penicillin-inhibited bacteria. By the chelation process, tetracyclines probably prevent the use of such essential ions as Mg, Fe, and Mn in enzyme systems essential for protein synthesis in growing bacteria. Evidence obtained in experiments with a C^{14}-labeling technique indicates that the inhibition site for tetracyclines is the surface of a catalytic enzyme which is probably involved in the metabolism of glutamic acid. The reversal of chlortetracycline inhibition of respiration in *Azotobacter* by magnesium salts offers evidence for chelation of certain enzyme-activating metal ions by this group of antibiotics.

The structural resemblance of some antibiotic compounds to essential metabolites suggests a possible mode of action through interference with specific steps of normal metabolism. In special cases, chloramphenicol appears to be antagonized by phenylalanine. Microbial inhibition by chloramphenicol may be explained as the result of blocked steps in protein formation; such blocking would allow the accumulation of D-glutamic peptides but not of peptides with the L-configuration.

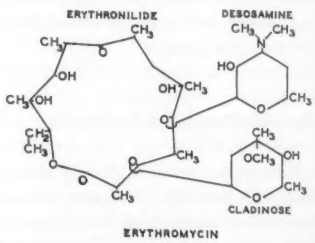
Studies on azaserine and 6-diazo-5-oxo-L-norleucine suggest interference with the incorporation of glycine and formate into nucleic acids. It has been proposed that streptomycin interferes with condensation of pyruvate and oxalacetate in the pathway of intermediary carbohydrate metabolism. The presence of an amino sugar in streptomycin, erythromycin, carbomycin, picromycin, and puromycin suggests the possible sig-



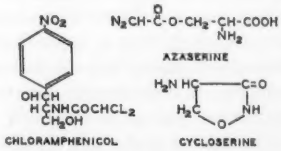
Depsidones occur commonly in certain species of lichens.



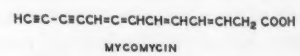
Tetracycline, chlortetracycline, and oxytetracycline are important broad-spectrum antibiotics.



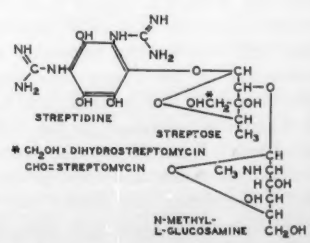
Erythromycin illustrates a macrolide ring structure with two satellite sugars, desosamine and cladinose.



Chloramphenicol bears a resemblance to B-phenylalanine, and azaserine and cycloserine are related to the amino acid serine.



Mycomycin is a polyacetylene.



Streptomycin is produced by some strains of the actinomycete *Streptomyces griseus*.

nificance of this structure in their mode of action. Recent evidence that sterols interfere with the inhibition of fungus growth by polyene antibiotics, such as filipin and amphotericin, provides an interesting hypothesis concerning their mode of action. The general body of evidence is compatible with the concept that biological action of antibiotics occurs by interference with enzymatic processes in susceptible organisms. Researches in this field have only scratched the surface of a gold mine of problems.

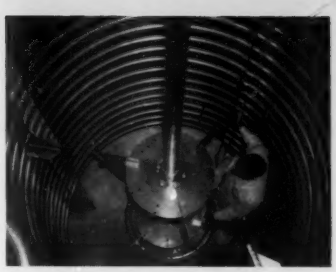
Current Trends

Numerous new compounds of remarkable organic structure and biological function continue to emerge from the large-scale research programs of government and industrial laboratories. One of the main activities for several years has been the screening of thousands of cultures isolated from soils of the world. The principal effort in antibiotic research is still directed toward the development of new therapeutic drugs. One reason for this is the need to fill gaps in the list of therapeutics; the other, the need to supplant old drugs with new ones in order to combat drug resistance.

The availability of new growth inhibitors for physiological studies helps in the making of numerous significant contributions to our knowledge about the life activities of living cells. The continuing research that is being done on modes of action of antibiotics is an important source of information for geneticists and biochemists. The molecular structure of an inhibitory compound may suggest a possible mode of action through resemblance to the structure of a known metabolite; sometimes the mode of action of a drug reveals much concerning incompletely understood pathways of metabolism. Knowledge about the structure and biochemical action of therapeutic agents, such as antibiotics, should eventually lead to the synthesis of effective "tailor-made" molecules for a more rational chemotherapy.

With the advent of so many kinds of drugs, clinicians are experimenting with combination therapy in the hope that mixtures of antibiotics may accomplish more good results than can be achieved with single drugs. Emergence of drug-resistant cells from a general population of drug-susceptible pathogenic organisms can create serious problems. The mechanism seems to be based upon spontaneous mutation of a few cells to resistance against, or dependence upon, a certain drug. The mutants subsequently respond by growing rapidly, and a new strain of pathogens emerges to challenge the patient's life and the physician's wisdom. Examples of drug resistance are found, all too frequently, among "hospital strains" of penicillin-resistant *Staphylococcus*. Other notorious offenders are the streptomycin-resistant tubercle bacteria, which may acquire resistance while the host is receiving low-level drug therapy. Among the various objectives behind the new trend toward polyantibiotics in medicine are the following: (i) to suppress different kinds of pathogenic microbes without specific diagnosis of the causal agents of disease; (ii) to prevent the emergence of drug-resistant strains; (iii) to achieve synergism; (iv) to avoid superinfection by opportunist fungi and other organisms; and (v) to arrest infections caused by mixed populations of unlike organisms.

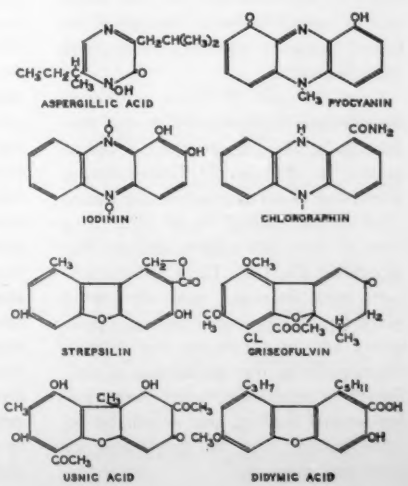
In the successful treatment of tuberculosis with a combination of streptomycin plus isoniazid or para-aminosalicylate, the emergence of resistant strains of tubercle bacteria is largely prevented. Another combination, designed to combat the overgrowth of pathogenic yeasts as secondary invaders, is found in Comycin, an antibiotic mixture which contains tetracycline phosphate plus the antifungal drug nystatin. A recent survey of the interactions of drugs against many kinds of pathogenic bacteria indicates that four predicted classes of interactions are actually found: (i) synergistic, (ii) additive, (iii) interfering, and (iv) negative. When two popular drugs were used alone and in dual combination with any one of 15 other antibiotics, the frequencies of occurrence of these types of interactions in results of antimicrobial tests were as follows: synergism, 24 percent; summation, 51 percent; antagonism, 3 percent; and no interaction, 22 percent.



Interior of a large fermentation tank used in producing antibiotic substances. [Courtesy of Parke, Davis and Co.]

A conspicuous recent trend is that of increasingly extensive manufacture of antibiotics for uses other than in human medicine. Large quantities of antibiotic drugs are used in veterinary medicine. Sometimes there may be some carryover of antibiotics from treated cows into the milk supply. Occasionally the antibiotic level in milk may be sufficient to interfere seriously in the microbial conversion of milk to cheese, or to cause allergic reactions in extremely sensitive persons.

Important applications of antibiotics have also been found in agriculture, as supplements in animal feeds. Practically all commercial poultry feeds contain low-level supplements of antibiotics such as penicillin, the tetracyclines, erythromycin, or bacitracin. Antibiotic-fed pigs, poultry, horses, and mink grow faster, require less feed, and show lower mortal-



(Top) Phenazine antibiotics of bacterial origin bear some resemblance to the mold product aspergillic acid. (Bottom) Numerous antimicrobial substances resemble usnic acid, commonly found in lichens.

ity rates than those not fed antibiotics. Of considerable interest is the use of antibiotics with sex hormones to produce better capons and superior beef. Growth in human beings seems also to be increased through low-level administration of certain antibiotics. Premature infants as well as young naval recruits have shown very significant gains in weight when kept on diets which included antibiotics as compared with controls maintained on the same diets without antibiotics. It is thought that these favorable effects may result from suppression of undesirable microbes, with consequent lessening of intestinal toxins and increase in the availability of essential nutrients and growth factors.

Antibiotics are being developed for a variety of other uses; these include everything from sprays for the arrest of bacterial and fungus diseases of plants to agents for increasing fecundity in cattle, agents for short-term preservation of foods, and so on. Preparation for slaughtering steers in some countries includes injection of antibiotic preservatives; these are carried into the tissues of the animal, so that later on the meat can be hung in warm rooms to bring about tenderization without spoilage. Extensive studies have shown the value of antibiotics in the preservation of fish, chickens, hamburger, vegetables, and other perishable foods. The propriety of such uses is under consideration by the Food and Drug Administration. In the control of certain plant diseases, antibiotics such as penicillin and streptomycin may act systemically throughout the plant and thereby have some advantage over conventional pesticides. Duramycin and actidione are examples of antifungal antibiotics employed for control of plant diseases. Antibiotic crop sprays are used to prevent blight in fruit trees and on bean plants and to control bacterial diseases of tobacco, tomatoes, cherries, lettuce, and other vegetables.

Over the past 15 years, antibiotics have become big business. The total production for all uses was over 2.7 million pounds in 1956 in the United States. The value of all antibiotics sold during 1956 in the United States came to a total of over 299 million dollars. The reports of the U.S. Tariff Commission show great increases, over the past 5 years, for antibiotics used as feed supplements and in veterinary medicine. It seems probable that production of antibiotics for agriculture, food preservation, and animal feeding, may eventually become greater than the production for medicinal purposes.

Areas of future research in the field of antibiotics will probably include methods for producing new genetic types of antibiotic-producing organisms, as well as ways for enormously increasing the yields of antibiotic substances through novel techniques. Methods may be found for developing new kinds of antimicrobial compounds with short half-life, suitable for the preservation of foods. The problems of finding more satisfactory controls of fungus diseases, tropical protozoa, severe virus infections, and the common cold offer challenges to the courageous investigator. New drugs will be needed to combat rare and dangerous diseases which may arise from microbial mutations in the future. Eradication of venereal diseases and other infections through a big public-health movement would be appropriate for an enlightened world civilization. Biological control through management of antibiotic soil microbes in nature might aid in bringing about extensive reduction of certain phytopathogenic fungi and undesirable nematodes, insects, and other agricultural pests. Discovery of microbial substances suitable for regulating fecundity in animals and man lies within the realm of possibility. Studies on the mode of action should lead to deeper understanding of the biochemistry of antibiotics and possibly to the rational synthesis of therapeutic agents far better than those that have become available through present trial-and-error methods.

Use and Abuse of Antibiotics

In this antibiotic age of chemotherapy problems arise out of the unwarranted widespread use of these powerful and sometimes dangerous drugs. The general population soaks up drugs like a huge sponge. On the basis of popular articles which they may have read, patients often demand access to the newest wonder drugs, through their physicians. The physicians, in turn, are subjected to high-pressure advertising which makes strong claims for (i) the potency of antibiotics; (ii) the synergistic power of antibiotics in combination therapy; (iii) the almost complete absence of side reactions; (iv) the ready absorption of these drugs by the body; (v) the prompt attainment of high blood levels; (vi) the slight extent of patient sensitivity; and, of course (vii) the applicability of antibiotics in most situations. It is not surprising to learn from a recent survey that in a typical small community in North Dakota, only 7.9 percent of the total population failed to receive antibiotics in a 5-year period. It is believed that the prescribed medication in this town was justified in less than half of the illnesses which were studied as case histories.

Indiscriminate use of antibiotics, and improper dosages, can cause harm through allergic reactions, blood dyscrasias, alteration of the normal flora, superinfections, emergence of resistant mutants, and fostering of persistent pathogens. As a result of nationwide surveys of severe reactions to antibiotic therapy, it was concluded that the highly potent antibiotics must be regarded as potentially dangerous drugs in the hands of uninformed persons. When these drugs are improperly used they can be dangerous; when properly used they are lifesaving. They should only be taken on the advice of a competent physician.

Antibiotics and the Public Health

Public health in the Western World has improved immensely over the 20 centuries which have passed since the time of the Roman Empire. In Caesar's Rome, the average life expectancy was about 22 years. Europeans of the Middle Ages could expect to reach, on the average, 33 years of age. In the United States today the newborn's statistical life expectancy is almost 70 years. Increased public health measures, improved diets, skillful surgery, better medication, and the general rise in standards of living all contribute to our living better and longer lives.

It is difficult to say how great have been the contributions of sulfa drugs and antibiotics to the public health. It seems quite clear, however, that marked reduction in mortality from many infectious diseases has occurred during the years since the introduction of "miracle drugs." This is true for meningococcal and streptococcal infections, influenza, pneumonia, tuberculosis, syphilis, and appendicitis, all of which yield to treatment with sulfonamides or antibiotics.

Before the antibiotic era, public health programs placed major emphasis on prophylaxis and the difficulty of therapy in venereal diseases. With the advent of penicillin, a remarkable reduction in the incidence of early syphilis has occurred. In New York City the decline in syphilis over 9 years following the 1946 postwar peak was from 78.5 to 7.7 per 100,000 of the population. Complete eradication of a disease is seldom achieved, and it is now important that attention be given

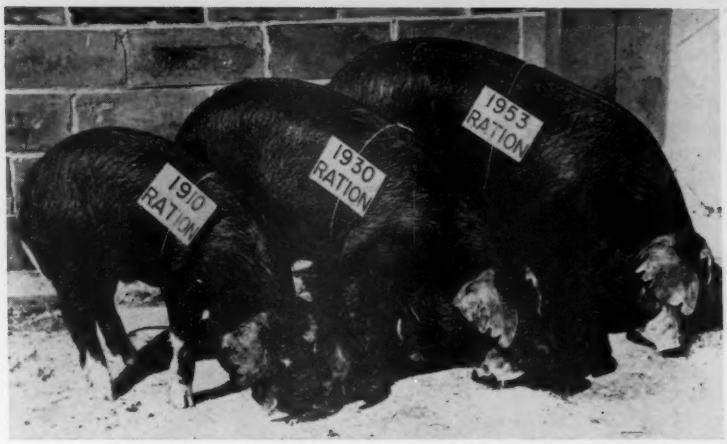
to gaining further ground in the conquest of syphilis. Gonorrhea also has yielded to penicillin therapy, but the emergence of drug-resistant strains of the pathogenic bacteria gives some grounds for concern. Here again, too much confidence in the merits of an established mode of therapy could lead to disaster.

As in the venereal diseases, so also in other common diseases a satisfying degree of control has been obtained by the use of antibiotics. Great changes have recently occurred in the management of tuberculosis. Whereas long periods of hospitalization were once regarded as necessary, now the tubercular patient can be trained to follow a prescribed regimen of combined therapy (with streptomycin and adjuvants) and sanitation during most of the convalescent period, spent at home. The serious effects of subacute bacterial endocarditis and rheumatic fever, and relapses of these diseases, have been greatly lessened by penicillin therapy and prophylaxis.

Recent estimates indicate that during the period from 1937 to 1952, approximately 1.5 million lives were saved through chemotherapy of the common infectious diseases mentioned above, exclusive of tuberculosis. When the data are brought up to date and other diseases are included, such as the enteric disorders, tuberculosis, and rickettsial diseases, the figures on conservation of human lives are much greater. Perhaps lifesaving with drugs has more than offset the losses sustained in the United States during the same period through wars and automobile accidents.

The impact of antibiotics and other wonder drugs on society is not wholly revealed in terms of improved medical care and prolongation of human lives. Modern miracle drugs are creating changes which lead to ponderable social problems. Some of these deserve to be mentioned here briefly. Since improved medication has tended thus far to conquer infections and steadily to increase the proportion of older people, the main medical challenges are being shifted to other areas. We will have to learn, more than ever, how to reduce disability, and how to make more effective use of the talents of healthy older people. This will come about more easily when science shows the way to extend the normal vitality of youth into what is now regarded as inevitable old age.

There are at present some distressing trends in our society which are aggravated by large-scale use of miracle drugs. Antibiotics today are keeping alive idiots who used to die under 12 years of age, as well as many thousands of persons who constitute a social liability. Half of the hospital beds in the United States are now occupied by mental patients, and there are tremendous numbers of mental defectives at large, making their contribution to the common gene pool. Insulin now prolongs the lives of numerous diabetic children, who marry and produce more victims of diabetes. Vitamin B_{12} gives patients with pernicious anemia ample time to propagate anemic offspring.



Advances made in livestock production are illustrated by the growth of pigs fed on rations typical of the years 1910, 1930, and 1953, respectively. The pig at left was fed yellow corn and minerals; the pig in the center received yellow corn, tankage, and minerals, and the pig at right was given a well-balanced ration fortified with vitamins and an antibiotic. "Miracle" drugs are bringing about great changes in agriculture. [Courtesy of Chas. Pfizer and Co., Inc.]

It is time to consider the possible untoward implications of an artificial system which fosters the survival of unfit germ plasm. Science has brought changes to society, and, as old problems become obsolete, new difficulties present their challenge.

One of the greatest problems of all time is destined to arise when miracle drugs and better nutrition are introduced into underdeveloped areas of the world. Almost three-fourths of the earth's human population live in countries where life expectancy is about 30 years, rather than 70. In many of these areas the birth and death rates are at least twice those of the United States. When infectious diseases in backward areas are overcome by the introduction of measures which have been found effective in the Western World, enormous numbers of Asians and Africans, as well as Americans, will live longer and have extended periods of fecundity. Perhaps these peoples can learn much, in advance, from the difficulties and experiences in Europe and North America. It is hoped so, because without knowledge and the right leadership, population pressures automatically produce poverty, famine, epidemics, and war. If birth control is felt to be desirable and if it is desired by people in overpopulated areas, what inexpensive, practical, and safe methods can be recommended to vast illiterate hordes of human beings?

Science has provided society with lifesaving drugs, and many times over it will need to redefine its objectives and seek solutions to difficulties as they arise. In the immediate future some problems of greatest concern to mankind will be cancer, degenerative ailments, mental diseases, population pressures, and management of our destiny in an all too complex society. Perhaps the methods of science, which generated knowledge about atoms and antibiotics, can continue to serve as Aladdin's lamp.

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The Raison d'Être of Secondary Plant Substances

These odd chemicals arose as a means of protecting plants from insects and now guide insects to food.

Gottfried S. Fraenkel

It has been stated that living plants, especially the flowering plants, furnish the food materials for fully half of the living species of insects. Every part of the plant may be eaten, but green leaves no doubt constitute the bulk of vegetable food material. Since leaves are the principal food of insects, and in most cases the only food, it is obvious that they must contain all the food materials which an insect requires. Yet we find that most insects that eat leaves are more or less selective in their choice of food plant. Insects may feed on only one species or on a few closely related plant species (monophagy), on a larger group usually confined within a certain plant family (oligophagy), or on a still wider group of plants (polyphagy), but they never feed on all plants. Is host selection in this group of insects governed by the nutritional superiority of the particular plant or region of the plant that serves as a food, or rather by the presence or absence of attractants and repellents in plants which are otherwise of more or less uniform food value?

The basic food requirements of all insects seem to be very similar and very much like those of higher animals. They include the "essential" amino acids, most of the vitamins of the B group, a sterol, and the physiologically important minerals. The nutritional requirements of plant-feeding insects are not known in most instances, but there is no reason to assume that they differ from those of other types of insects which have been more extensively studied. These basic requirements of insects concern substances which occur in *all* living cells, including, of course, those of plant tissues. Insofar as the occurrence of these substances is concerned, the composition of all leaves is very much alike, and there is little reason to suppose that differences in chemical composition with respect to the "primary" substances (which occur in all living matter) can be responsible for the choice of food plant on the part of the insect (*1*).

Plants also contain a vast array of what have been called "secondary" plant substances (*2*). These may be conveniently grouped as glucosides, saponins, tannins, alkaloids, essential oils, organic acids, and others, many thousands of which have been described in the literature. Their occurrence is sporadic but may be specific for families, subfamilies, and genera and sometimes even for species or subspecies. Their role in the metabolism of plants has never been satisfactorily explained, but in view of their sporadic occurrence and of the differences in their chemical constitution, it is almost inconceivable that they play a function in the basic metabolism of plants. For the same reasons, it is also highly improbable that they are of nutritional importance for the insects in the same sense as the "primary" substances are—namely, that they are metabolized and utilized in tissue synthesis.

It is suggested that the food specificity of insects is based solely on the presence or absence of these odd compounds in plants, which serve as repellents to insects (and other animals) in general and as attractants to those few which feed on each plant species. The immense variety and number of compounds concerned thus corresponds to the equally immense variety of specific nutritional relationships between insects and plant hosts. The compounds concerned need not play any role in the basic metabolism of either the plant or the insect, since they serve merely as trigger substances which induce, or prevent, uptake of the true nutrients. Most, if not all, secondary plant substances possess characteristic odors or tastes and thus may elicit sensory reactions to the food. In contrast, most of the important nutrients, like proteins, starch, fats, vitamins, or minerals have little or no taste or smell, at least not at the levels at which these nutrients are present in plants.

It is suggested, then, that leaf-feeding insects could develop equally well on any leaves, provided they ate enough of them. We must assume that early in their evolution plants developed the characteristics which made them unpalatable to the rising multitude of insects. The unpalatability was accomplished by the production of the vast array of chemical compounds which characterize specific taxonomic groups of plants. In fact, the appearance of the flowering plants in the early Cretaceous coincides with the various morphological and physiological adaptations in both insects and plants which characterize the interdependence between the insects and the flowering plants. This reciprocal adaptive evolution which occurred in the feeding habits of insects and in the biochemical characteristics of plants forms a striking parallel to the better understood relationship between the shape, color, and scent of flowers and the sensory responses of insects. It is common knowledge that the pigments and flavoring substances of blossoms owe their existence solely to their functions as attractants for insects. Is it less logical to assert that the secondary substances in plants exist solely for the purpose of repelling and attracting insects?

Had the plants been entirely successful in developing their chemical protection against insects, there would be no insect problem in agriculture. In fact, however, insects on their part responded to this chemical control of the plant. A host preference arose when a given insect species, by genetic selection, overcame the repellent effect of such a material, thereby gaining a new source of food. This led to a situation where further selection produced new species or genera of insects that require the former repellent as an attractant to induce feeding.

To establish the ecological relationships between insects and secondary plant substances, the following points should be considered or proved. (i) The active substance should be isolated and

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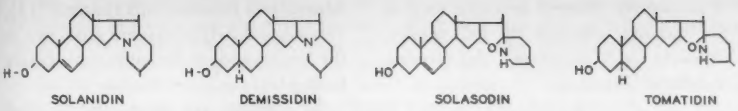


Fig. 1. Chemical structures of four aglycons.

identified. (ii) The isolated compound, if an attractant, should induce feeding when applied to leaves which are not commonly accepted as a food by a certain insect, or when incorporated in neutral media like filter paper or agar jelly. (iii) Members of plant families other than those which commonly contain the attractant in question should be acceptable if they normally contain this compound. (iv) A quantitative relationship should exist between the concentration of an attractive substance and feeding response. (v) A compound which serves as a repellent in a particular insect-plant relationship should, when incorporated in the normal food plant, make it unattractive. (vi) A plant may be attractive and at the same time poisonous, either through possessing separate attractive and poisonous compounds or through presenting these two effects in one and the same compound.

In the following paragraphs the situation as it has emerged for some of the principal plant families—Cruciferae, Umbelliferae, Solanaceae, Leguminosae, Moraceae, and Gramineae—will be discussed.

Cruciferae

All members of this family contain glucosides with a mustard oil as the aglycon; some of these glucosides, like sinigrin and sinalbin, are widely distributed. Cruciferae have a very characteristic fauna of lepidoptera, flea beetles, and aphids. The first detailed description of a chemical insect–host-plant relationship concerned the work of Verschaeffelt (*3*) on the white cabbage butterflies *Pieris brassicae* and *P. rapae,* which feed almost exclusively on members of this family. Forty years later, his work was largely confirmed and was extended to the diamondback moth *Plutella maculipennis* by my collaborator, Thorsteinson (*4*). These insects feed exclusively on Cruciferae and a few species of other plant families which contain similar glucosides. Many other plants were eaten by *Plutella* but only after they had been treated with sinigrin or sinalbin. A quantitative relationship exists between the glucoside content of the food and the feeding response of *Plutella.*

Table 1. The inhibitory action of a number of alkaloids from Solanaceae on potato beetles, in relation to the composition of the sugar component and the structure of the aglycon (Fig. 1). [After Schreiber (*8*)]

Degree of inhibition	Glycoalkaloid	Plants of origin	Sugar component	Aglycon	Double bond in aglycon
++++	Tomatin	*L. esculentum*	Tetrasaccharide with xylose	Tomatidin	Absent
++++	Demissin	*S. demissum*	Tetrasaccharide with xylose	Demissidin	Absent
++++	(Tetrasid)	*S. polyadenium*	Tetrasaccharide with xylose	Tomatidin	Absent
+++	(Tetrasid)	*S. schreiteri* and *S. punae*	Tetrasaccharide with xylose	Solanidin	Present
++	(Triosid)	*S. polyadenium*	Trisaccharide with xylose	Tomatidin	Absent
++	Solacaulin	*S. acaule* and *S. caulescens*	Trisaccharide with xylose	Solanidin	Present
++	β-Tomatin*		Trisaccharide without xylose	Tomatidin	Absent
++	β-Demissin†		Trisaccharide without xylose	Demissidin	Absent
+	Dihydro-α-solanin‡		Trisaccharide with rhamnose	Demissidin	Absent
–	α-Chaconin	*S. chacoense* and *S. tuberosum*	Trisaccharide with rhamnose	Solanidin	Present
–	Solamargin	*S. nigrum, S. sodomeum, S. aviculare,* and *S. auriculatum*	Trisaccharide with rhamnose	Solanidin	Present
–	Solasonin	*S. nigrum, S. sodomeum, S. aviculare,* and *S. auriculatum*	Trisaccharide with rhamnose	Solanidin	Present
–	α-Solanin	*S. tuberosum*	Trisaccharide with rhamnose	Solanidin	Present

* Prepared by partial hydrolysis of tomatin; † Prepared by partial hydrolysis of demissin; ‡ Prepared by hydrogenation of solanine.

Umbelliferae

This family is characterized by the presence of essential oils, many of which are known as constituents of spices. According to the investigations of Dethier (*5*), *Papilio ajax* was found feeding on 18 different plants of this family, containing any of the following crude oils: carrot, caraway, anise, coriander, celery, and angelica. Pieces of filter paper treated with such oils or with pure constituents of oils, such as carvone (from caraway), methyl chavicol (from anise), or coriandrol (from coriander), were also attacked. Methyl chavicol is also contained in certain nonumbelliferous plants (Rutaceae: *Dictamnus fraxinella*; Compositae: *Solidago* sp. and *Artemisia dracunculoides*) which are eaten by this insect.

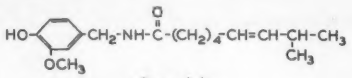
Leguminosae

The Mexican bean beetle, *Epilachna varivestis,* feeds almost exclusively on plants of the genus *Phaseolus* but has, in recent years, become increasingly adapted to the soybean in the United States. It never feeds on *Vicia faba.* Evidence points to the effect of a glucoside of the nature of a triterpenoid saponine as the attractive factor. This compound has been concentrated but not yet isolated (*6*).

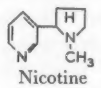
Solanaceae

The very extensive work of Kuhn, Schreiber, and others on the structure and occurrence of glycoalkaloids in Solanaceae and their effect on the potato beetle *Leptinotarsa decemlineata* has been summarized by Kuhn and Löw (*7*) and by Schreiber (*8, 9*). These alkaloids, contrary to former expectation, do not make the plant attractive to the beetles but are the agents which render a species of Solanaceae repellent or toxic. The

compound which makes the potato plant attractive to *Leptinotarsa* has never been identified. Glycoalkaloids of related structures occur in Solanaceae in an astonishing diversity of structures, those occurring in the common potato plant, solanine and chaconine, being harmless and of no apparent effect on the potato beetle, and those in other plants—for example, in tomato, *Solanum demissum, S. chacoense,* and tobacco—being repellent and sometimes toxic. Schreiber (*8*) attributed the adversely acting properties to the lack of the double bond in the aglycon, to the tetra- (as against the tri-) saccharide component, and to the presence of xylose (Table 1 and Fig. 1). The following were recognized as repellent compounds in other Solanaceae: tomatine (in tomato), the "leptines" in *S. chacoense* (Kuhn in *10*), soladulcin and *S. dulcamare* (*8*), a tetrosid in *S. acaulia* (*8*) (Table 1), and compounds of an entirely different structure, such as the burning principle of red pepper, capsaicin (in *S. capsicum*), and nicotine in tobacco (*8*).



Capsaicin



Nicotine

One of the most striking demonstrations of the repellent effect of nicotine on the potato beetle is presented in certain grafting experiments. It is well known that nicotine in tobacco is synthesized in the root. A tobacco plant which is grafted on a potato root is free of nicotine and is eaten by *Leptinotarsa.* Conversely, a potato plant grafted on a tobacco root becomes fully resistant (*11*). *Petunia* and *Salpiglossis* are attractive to the beetles and, at the same time, highly toxic (*12*).

By contrast, the most efficient food plants of the tobacco hornworm *Protoparce sexta* are tobacco and tomato. This insect feeds widely, however, and almost exclusively, within the family Solanaceae (Table 2). A substance attractive to this insect and found in plants of this family, of the nature of a glucoside but not containing an alkaloid, has been isolated but not yet identified. This substance also appears to be attractive to the potato beetle. *Petunia* is also eaten by this insect, with toxic effects (*13*).

Table 2. Feeding and growth responses of the tobacco hornworm to various plant materials. [Data from Yamamoto (*13*)]

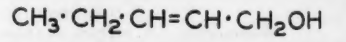
Plant	Common name	Acceptability*	Support of growth†
Group A. Solanaceous plants			
Lycopersicon esculentum	Tomato	+++	+++
Datura stramonium	Jimson weed	+++	+++
D. stramonium tatula		+++	+++
D. innoxia		+++	Not tested
D. wrightii		++	++
Lycium halimifolium		+++	+++
Nicotiana tabacum	Tobacco	+++	+++
N. affinis		+++	+++
N. rustica		+++	+++
N. suaveolens		+++	+++
Solanum nigrum	Black nightshade	+++	+++
S. rostratum	Buffalo bur	+++	+++
S. carolinense	Horse nettle	+++	+++
S. tuberosum	Potato	+++	+++
S. dulcamara	Deadly nightshade	+++	+++
S. melongena	Eggplant	++	Not tested
Physalis virginiana	Ground cherry	+++	+++
P. heterophylla		+++	+++
P. alkekengi		+++	Not tested
P. ixocarpa	Tomatillo	++	Not tested
Nicandra physalodes		+	+
Petunia hybrida	Petunia	+++	– –––
Capsicum annuum	Green pepper	++	++
Browallia americana		+++	Not tested
Brunfelsia americana		+++	Not tested
Group B. Nonsolanaceous plants			
Lactuca sativa (Compositae)	Lettuce	++	+
Brassica oleracea, var. *capitata* (Cruciferae)	Cabbage	++	+
Phaseolus spp. (Leguminosae)	Green bean	–	–
Ipomea purpurea (Convolvulaceae)	Morning-glory	–	–
Ulmus americana (Ulmaceae)	Elm	–	–
Quercus spp. (Fagaceae)	Oak	–	–
Catalpa spp. (Bignoniaceae)	Catalpa	–	–
Morus spp. (Moraceae)	Mulberry	–	–
Plantago spp. (Plantaginaceae)	Plantain	–	–

* (+++) Readily acceptable; (++) acceptable after 2 hours of contact; (+) acceptable after 8 hours of contact; (–) rejected; (– – –) toxic effect.
† (+++) Normal growth; (++) slow growth and low mortality; (+) slow growth and high mortality; (–) nonsupport of growth; (– – –) premature death.

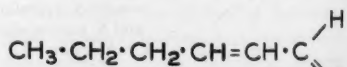
Moraceae

The relation of the silkworm *Bombyx mori* to its food plant, the mulberry tree, *Morus alba,* has long been claimed as one of the most striking examples of monophagy. Yet this insect is known to be capable of feeding not only on a number of other Moraceae, such as several species of *Cudrania, Maclura, Broussonetia,* and *Ficus,* but also on several Compositae, such as lettuce, dandelion, and *Scorconera.* A great deal of evidence to this effect is presented by Tanaka (*14*). Hitherto emphasis has been placed on the fact that the silkworm feeds on substitutes for *Morus* for only short periods, or that the various substitutes are otherwise inferior to *Morus.* From the point of view of food specificity, however, all positive reactions of the silkworm to other plants, independent of the ultimate result, are highly revealing.

Evidence has been presented by Watanabe (*15*) to the effect that silkworms are attracted to many different plants from a distance; some of these (such as mulberry) are then eaten, while others (soybean and tea) are not, while other plants, such as fig and lettuce, though not so attractive from a distance, are often eaten to some extent. The attractive principle has been isolated from a steam distillate of many plants and identified as β-γ hexenol and α-β hexenal (*16*). These compounds are



β-γ HEXENOL (LEAF ALCOHOL)



α-β HEXENAL

Table 3. Degree of feeding by larvae of the silkworm *Bombyx mori* on various plants of the family Moraceae and the effects of such feeding with respect to growth and survival of the larvae. The degree of response is expressed by a graded number of plus signs and minus signs: (++++) performance on mulberry leaves (maximum response); (+) a small but definite response; (±) feeding hardly noticeable; (−) no feeding, responses as of starving insects; (−−) survival time shorter than that for starving insects. L, reported in the literature; F, my own experiments (unpublished).

Name of plant	Feeding	Growth	Survival	Reference
		Urticaceae		
Urticaceae	+	?	?	L
Urtica procera	++	−	−	F
		Moraceae		
Morus alba	++++	++++	++++	L, F
Maclura aurantiaca	++++	++++	++++	L, F
Broussonetia kazenoki	++++	++	+	L, F
B. papyrifera	+	−	−−	F
Cudrania triloba	++++	++++	++++	L, F
C. javanensis	+++	+++	+++	L, F
Ficus carica	++	+	−−	L, F
F. elastica	++	+	−	F
F. erecta	±	−	−−	F
F. wightiana	−	−	−	F
F. pumila	++	+	−−	F
F. retusa	+	+	−−	F
F. hirta	++	+	−	F
Humulus lupulus	−	−	−	F
H. japonicus	−	−	−	F
Cannabis sativa	−	−	−	F
Dorstenia contrajerva	−	−	−−	F
		Ulmaceae		
Ulmus sp. (*parvifolia?*)	++++	++	+	F

widely distributed in leaves from various sources and may well serve also to attract other leaf feeders to their food. However, the specificity of the food plants of the silkworm seems to be due to other types of compounds, recognized rather by taste than by their smell, which are typical of the plant family Moraceae. Table 3 summarizes the effect on the feeding, growth, and survival of *Bombyx* of a number of plants of this family (*17*). In this table, five plus signs indicate the effect of the proper food plant *Morus alba*, and the responses of *Bombyx* to other plants are indicated by a series of plus or minus signs. One minus sign indicates that the plant had not been touched at the time of the insect's death, and that death occurred after the same length of time as in an insect kept without food. It may be seen that silkworms feed relatively well on the American tree *Maclura aurantiaca* (Osage orange), on *Broussonetia kazenoki*, and on *Cudrania triloba* and *C. javanensis*. Development of *Bombyx* from the egg to the adult on all these plants has been reported, though growth was slower and the period of survival was shorter than for silkworms that fed on *Morus*. Relatively profuse feeding also occurred on several species of *Ficus*, though for only short periods. Indeed, on a number of the plants eaten, death occurred sooner than it did in insects that had been starving (as indicated by two minus signs in Table 3). This suggests that an attractive principle is widely, if not generally, distributed within plants of the family Moraceae, but that the amount of feeding and the effect on growth and survival are limited by the simultaneous presence of repellent or toxic principles. On the edible fig, for instance, or on the paper tree *Broussonetia papyrifera*, *Bombyx* feeds eagerly for a few days and then dies. The fact that the larvae feed on Compositae may indicate the absence of a repellent in such plants, but feeding only occurs when the larvae are hungry and is not sustained for long periods From the point of view of phylogenetic distribution of specific plant substances, it is highly revealing that profuse feeding also occurs with the family Ulmaceae, and that similar observations have also been made with respect to members of the Urticaceae. In the natural classification of plants, Ulmaceae, Moraceae, and Urticaceae together form the order Urticales.

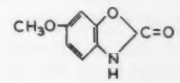
The effect of repellents on the feeding reactions of *Bombyx* is strikingly illustrated by the work of Torii and Morii (*18*). Extirpation of the maxillae resulted in feeding on several plants (for example, cherry and cabbage) which were normally not touched by the insects; obviously this was because they no longer tasted a repellent substance.

None of the attractive or repellent or toxic principles which occur in moraceous plants have so far been identified.

Gramineae

The European corn borer *Pyrausta nubilalis* and the rice stem borer *Chilo suppressalis* are among the few plant-feeding insects which have hitherto been grown on a synthetic diet with some measure of success. However, development was far from optimal in the absence of additional leaf fractions—a fact which strongly points to the importance of attractant substances. The isolation of such a substance from rice plants has now been reported by Munakata *et al.* (*19*). The method of assay consisted of trapping larvae of the rice stem borer in small glass tubes containing the substance. This compound was provisionally identified as an aliphatic unsaturated ketone and named orizanone.

Nothing is yet known about the nature of the attractant for the corn borer. However, evidence of very interesting consequences has been presented about limitation of feeding caused by resistance factors. One fat soluble factor (resistance factor A) which is responsible for the high mortality on young corn plants of pretassel stage has been identified as 6-methoxybenzoxazolinone (*20*) a com-



pound, incidentally, which was also isolated from rye plants and which prevented the growth of the rye plant rot *Fusarium nivale*. Varietal differences in resistance to borers at this stage of growth are positively correlated with varietal differences in concentration of resistance factor A in the plant (*21*). The resistance tends to break down when the plant tassels. The tassel, which is a favorite feeding site, contains little or none of the resistance factor. The occurrence of two other, water-soluble, resistance factors, B and C, has been inferred, but nothing is yet known about their nature.

The oriental migratory locust *Locusta migratoria* has long been regarded as an almost indiscriminate plant feeder. Yet, according to recent investigations by Chin (22) (which I can corroborate), in nature it feeds exclusively on about 20 species confined to the families Gramineae and Cyperaceae. In the laboratory, in the absence of grasses, it also grows on cabbage or soybean, but slowly,

as a result of diminished feeding. It appears that sensory discriminations serve as the determining factors in selective feeding, but that once the food is ingested, digestion goes on without much regard to difference in food type.

Members of the family Gramineae are characterized by the occurrence of silica in the leaves. It has frequently been assumed that the presence of silica deters animals from feeding on grasses. Isley (*23*) demonstrated that the mandibles of species of grasshoppers which fed on grasses were more highly sclerotized than those of grasshoppers which did not. Sasamoto (*24*), in a series of papers, has shown a correlation between the silica content of rice plants and the degree of attack by the rice stem borer *Chilo suppressalis* Walker. In these studies the silica content of the plants was increased by applying silica to the soil. The leaves of silicated plants caused abrasions on the mandibles of insects which fed upon them.

Conclusion

The examples cited of insects specific to plants of the families Cruciferae, Umbelliferae, Leguminosae, Solanaceae, Moraceae, and Gramineae clearly demonstrate the function of secondary substances in these plants as means of repelling or attracting insects. The fact that, so far, only insects have been discussed does not imply that of all the organisms which depend on plants for nutrition, insects are the only group of importance with respect to the particular phenomenon under discussion. In fact, the first comprehensive statement on this topic, by Stahl (*25*), originated from a consideration of the relation between insects and snails, and there is every reason to assume that other organisms, such as mites and, in particular, the vast array of pathogenic and commensal microorganisms, from bacteria to fungi, which inhabit plants, are affected in a similar way by secondary plant substances. Insects, however, not only provide at present the best known instances but also in all probability are, of all organisms, the ones that play the principal causative role in this relationship.

This relationship between secondary plant substances and insects does not come as a surprise to the entomologist and ethologist, who have always been impressed by the power of discrimination of the chemically stimulated senses of insects, not only in the areas under discussion here but also in the reactions of insects to animal hosts of all kinds, in the mutual recognition of the sexes, and in the selection of egg laying sites. But it must come as a surprise to the plant physiologists, biochemists, and organic chemists, who for generations have been dealing with these substances and who have been entirely in the dark about the proper function of these compounds for the plant—indeed, about their *raison d'être*. In the innumerable books and papers which have been written on glucosides, tannins, alkaloids, and essential oils, one searches in vain for a comprehensible and comprehensive statement about the true function of these compounds and their origins in the phylogeny of plants, And yet, the views here expressed were stated over 70 years ago with great forcefulness by the German botanist Stahl (*25*) in a treatise on the protection of plants against snails. They were first successfully put to the test by Verschaeffelt (*3*) in his already cited investigation on *Pieris* butterflies and Cruciferae and subsequently restated in various perspectives by Dethier (*26*) and by me (*27*). There were full discussions of the insect–host-plant relationship in two symposia held in recent years—that on the "Physiological Relations between Insects and their Hostplants," at the International Congress of Entomology, held in Amsterdam in August 1951 (*28*), and that on "Insect and Foodplant," held in Wageningen, the Netherlands, in 1957 (*29*).

To summarize, it would be difficult to find a more lucid and concise statement than the following sentences, which close Stahl's pioneering article (*25, 30*):

"We have long been accustomed to comprehend many manifestations of the morphology [of plants], of vegetative as well as reproductive organs, as being due to the relations between plants and animals, and nobody, in our special case here, will doubt that the external mechanical means of protection of plants were acquired in their struggle [for existence] with the animal world. The great diversity in mechanical protection does not appear to us incomprehensible, but is fully as understandable as the diversity in the formation of flowers. In the same sense, the great differences in the nature of chemical products [*Exkrete*], and consequently of metabolic processes, are brought nearer to our understanding, if we regard these compounds as means of protection, acquired in the struggle with the animal world. Thus, the animal world which surrounds the plants deeply influenced not only their morphology, but also their chemistry."

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30. Translation from the German by G. Fraenkel.

William Moffitt, Theoretical Chemist

In the sudden death of William Moffitt on 19 December 1958, theoretical chemistry lost one of its most brilliant practitioners. The loss is the more acute, occurring as it did when Moffitt was only 33. During the past decade he had been foremost in recognizing and solving a succession of major problems relating to molecular structure. The very consistency of this performance had led to the expectation that we would witness a lifetime of intellectual conquest in keeping with the best traditions of British and American science. This is not to be. However, our grief must be tempered by the richness of what he has left behind. His published works stand as a living monument that will be admired and consulted as long as men seek to understand the molecules of which their world is made. His life as he lived it will remain vivid in the memories of his friends and students, for the boundless joy he found in pure science; for the openness of his personality, which was free of banality; for his adherence to high intellectual standards uncompromised by fashion; for his warmth and charm and wit; and for the way he could make the English language sing.

William Moffitt was born in Berlin of British parents. He entered New College, Oxford, in 1943 and received the B.A. degree in 1946 and the D.Phil. degree in 1948. During work for his doctorate with Professor C. A. Coulson, his interests became channeled into quantum mechanics and its application to molecular structure. His constant concern with making theoretical knowledge meet the test of elucidating or predicting the behavior of real molecules could be seen in his first work, in which he demonstrated that existing quantum mechanical concepts could satisfactorily explain the structure of some free radicals, conjugated hydrocarbons, and dyes, as well as the state of strain existing within certain molecules.

William Moffitt

For four years after leaving Oxford he lived in London, where, as a member of the staff of the British Rubber Producer's Research Association, he pursued with great freedom and independence a number of original lines of theoretical investigation through which he reached scientific maturity. These included modifications of the molecular orbital theory to illuminate the structure of carbon monoxide, carbon dioxide, and certain compounds of phosphorus and sulfur. The culmination of this work was in the development of a new conceptual view of the electronic structure of molecules, known as the "atoms-in-molecules" concept, which has been widely adopted. In the hands of the author it led to his prediction of two new states of the oxygen molecule, which were later confirmed experimentally.

Early in 1953 Moffitt came to Harvard University as assistant professor of chemistry, and he was promoted two years later to associate professor. His first work in his new environment dealt with the quantitative prediction of the absorption of aromatic hydrocarbons. Thereafter his work fell into two general areas. In one he delved into well-worked areas of molecular optics and emerged with new findings that created a renaissance—first in showing how dichroism and optical rotation could be used to solve basic problems in the crystal field theory of inorganic complex ions, then in showing how the dispersion of optical rotativity, the Faraday effect, and the Kerr effect were related to spectroscopic properties. These researches have set in motion experimental investigations all over the world and have been influential in the structural determinations of proteins and, most recently, of steroids. Parallel to this he continued his specific interests in the electronic structure of molecules. In this he found the way in which certain electronic motions can couple with nuclear modes, and by this means he was able to solve several important and puzzling stereochemical and spectroscopic problems.

Moffitt's ability to make significant contributions stemmed from his mastery of mathematics and physics on the one hand and his wide and detailed knowledge of many different areas of modern chemistry on the other. His genius lay in his ability to delineate what could be solved and what could not, and what was worth solving from what was not. This having been determined, he would seek his goal with all the force of his robust personality until the solution was found and formulated with that combination of rigor and simplicity and elegance that was the trademark of all that he did.

Shortly before his death, which resulted from a heart attack that occurred while he was playing squash, he unknowingly wrote his own epitaph. "I am a scientist because I enjoy being one more than anything else. I find nothing so satisfying as trying to form convincing bridges between the elegant and elementary principles of modern physics and the much more complicated and yet empirically well-characterized situations encountered in chemistry. The exercise is not unrewarding, since a successful theory most frequently has fruitful applications. Perhaps because I do no experimental work, I aim to be useful."

Moffitt was a fellow of the American Academy of Arts and Sciences, associate editor of the *Journal of Chemical Physics* and the *Journal of Molecular Physics*, and a member of the American Chemical Society and the American Physical Society.

PAUL DOTY

Harvard University, Cambridge, Massachusetts

News of Science

World Health Year Plans Advanced

Secretary of Health, Education, and Welfare Arthur S. Flemming recently urged that a great world crusade of health for peace be launched through the International Health Year, a health study period that has been proposed to parallel the International Geophysical Year of 1957–58. His appeal was made at the opening dinner session of the second National Conference on World Health, which was held in Washington, 7–9 May, under the auspices of the National Citizens Committee for the World Health Organization.

Milton S. Eisenhower, president of Johns Hopkins University, was chairman of the conference, which brought together leaders of Congress and of the executive branch of the Federal Government and representatives of organizations and industrial companies interested in health and international relations. The principal objectives of the conference were to discuss the international health legislation now before the Congress, to appraise the value of international health programs in developing habits of cooperation among nations, and to outline a future International Health Year.

Stevenson First to Propose

The original suggestion for such a Year came from the former Governor of Illinois, Adlai Stevenson, in an address delivered 8 June 1958 at Michigan State University. On 9 June, Senate majority leader Lyndon B. Johnson (D-Tex.) had Stevenson's remarks printed in the *Congressional Record*. That same day Senator Hubert H. Humphrey (D-Minn.) commended the address on the Senate floor. Humphrey has been the dedicated champion of the proposal ever since. In mid-August 1958 Senate Concurrent Resolution 99, which he had introduced, was passed. It said: "The President of the United States is hereby invited to extend to the other nations of the world, through the World Health Organization, and related organizations, an invitation for the designation of representatives to meet and discuss the feasibility of designating an International Health and Medical Research Year, at such early date as adequate preparations can be made, or of other methods of developing such intensive international cooperation in the field of health as will lead toward the discovery and exchange of the answers on coping with major killing and crippling diseases which afflict mankind."

Some 2 weeks later, on 6 September, the Ukrainian Soviet Socialist Republic presented to the United Nations General Assembly a resolution for the organization of an International Public Health and Medical Research Year. Under the auspices of 22 nations, including the United States, the resolution was approved in December 1958.

Next, in January 1959, the Health Year was considered by the World Health Organization's executive board, which asked the WHO director general to prepare plans for the proposed Year for presentation at the 12th WHO assembly. That assembly is now in session in Geneva.

Forum Defines Health Year

Participants in the recent National Conference on World Health in Washington included most of the U.S. delegation to the current Geneva meeting. Some of this group played an active part in a forum on the International Health Year that was held under the chairmanship of James E. Perkins, managing director of the National Tuberculosis Association. The panelists were Albert W. Dent, president of Dillard University; James E. Hundley, special assistant for international health at the National Institutes of Health; and Julius N. Cahn, project director of the International Health Study of the Senate Committee on Government Operations.

Cahn, who has been working closely with Senator Humphrey on the Health Year, was the first speaker. His statement, which represented the views of many of the discussants heard later, presented seven points formulated to help assure the success of the Year.

1) The program should be based on the individual nations' own felt needs.

2) There should be strong cooperation by national governments, but basically—as in the International Geophysical Year—success will be dependent on private initiative, the initiative of the complex of private scientific and other organizations.

3) The Year will require the enthusiastic support of the medical profession everywhere, but it should be broad enough in concept to allow the fullest possible contribution by laymen as well.

4) All the life sciences must be involved.

5) In addition to WHO, the other health-oriented international organizations that are allied with the U.N. should participate, such as the United Nations Educational, Scientific, and Cultural Organization, the International Labor Organization, the Food and Agriculture Organization, and the United Nations Children's Fund.

6) Participants should be willing to undertake bold experiments in the health field. There must be an effort to establish new models of experimental collaboration, new approaches, new techniques.

7) Provision should be made for continuation of the projects started during the International Health Year so that the momentum gained during the period will be sustained in years to come.

Cahn then mentioned specific areas that ought to be involved in the project. He emphasized that the most important single IHY program should be the expansion of epidemiological services throughout the world and the strengthening of data concerning the distribution of various diseases. Another great need that could be met by IHY would be that for increased training of professional and nonprofessional medical personnel; this would include increased exchange of scientists in the health field and the organization of international seminars. Further, particular attention must be paid to the problems of providing the world's supply of water and food. The various nations should attempt to single out one or two diseases for a specific campaign of eradication. Examples given, in addition to malaria and smallpox, currently the subject of control programs, were tuberculosis, cholera, and schistosomiasis. In like manner, certain important problems should be singled out for intensified research. Cahn suggested as possibilities radiation and air pollution. There should be health education of the masses. And finally, certain broad projects should be selected for emphasis during the IHY that would allow citizens to do things for themselves so that they would feel a sense of participation. For example, in the United States this might be achieved through a special campaign to increase the number of women who take annual diagnostic tests for uterine cancer.

The next panelist to present his views was James Hundley, who proposed that

each country hold a meeting to reach agreement regarding the final plan for the Year for that particular country. He pointed out that the Year has two elements: an international cooperative element and the individual programs of the various nations.

With regard to a possible national plan for this country, Hundley made several specific suggestions that fell into three classes: research projects of special importance to the United States, research on problems as important to other countries as to the United States, and research that would be of benefit almost entirely to other countries.

Albert W. Dent was the final panelist to speak. He stressed the importance of citizen participation in the programs selected and the need to evolve better techniques in educating and motivating people to participate in health programs, such as in the program of vaccination against poliomyelitis and that of tuberculosis control. He pointed out that public apathy has developed with regard to both of these diseases.

Plan Being Considered by WHO

In the general discussion that followed the panelists' presentations, H. van Zile Hyde of the U.S. Public Health Service, and U.S. member of the WHO executive board, outlined briefly what the director of the World Health Organization is proposing with respect to the International Health Year at the current World Health Assembly in Geneva. The object of the Health Year, as presented by the director general's report, is "to stimulate, primarily on a national basis, the intensification of international cooperation in carefully selected aspects of health and of medical research." This will involve the intensification of field activities in the control or eradication of specific diseases and the intensification of research related to WHO's growing program. Examples of field activity mentioned by the director general include renewed emphasis on malaria and smallpox eradication and installation of piped water supplies. As examples of fields for increased research, he cited cancer, cardiovascular diseases, and virus diseases. The director general further suggested that national committees be formed throughout the world to stimulate interest in and to plan for the IHY.

The Washington forum carried this idea further by proposing that as a framework for the International Health Year a series of national assemblies be held, dealing with health problems in the respective countries, and that the year might close with a climatic congress held in connection with the World Health Assembly in the spring of 1963. The forum session ended with unanimous passage of a resolution that read: "Forum No. 2 recommends urging the U.S. Delegation to the Twelfth World Health Assembly to support in the Assembly the designation of an International Health Year, to start in 1961, and further recommends that the National Citizens Committee for the World Health Organization, the Department of Health, Education, and Welfare, the Congress, and other groups give all possible support to the project."

Under last September's U.N. resolution, WHO has been invited to report on the International Health Year to the U.N.'s Economic and Social Council at its 28th session this July, and to the General Assembly at its 14th session, which will begin in September.

In the United States, Congressional sources confidently predict that adequate funds will be provided for the IHY once the appropriate scientific authorities, governmental and nongovernmental, have developed specific programs for the Year. As in the case of the International Geophysical Year, bodies such as the National Academy of Sciences, the National Science Foundation, and the Department of Health, Education, and Welfare are being asked to draft the framework for the International Health Year program that will eventually be submitted to Congress for consideration.

Secretary Arthur S. Flemming addresses the National Conference on World Health.

Reports Disagree on Radiation Hazards

The issue of radiation dangers continues to stir wide controversy among Congressmen, scientists, and journalists in Washington. A flurry of reports, often contradictory in their conclusions, is behind the current flare-up of the radiation issue that first received public attention during the 1956 presidential campaign.

Publication last month of a report by the National Committee on Radiation Protection and Measurement started the controversy. This report made substantial downward revisions in the committee's previous estimates of the dangers posed by strontium-90. Another report, issued by an international group, made a contrary recommendation and suggested that the current "permissibility" limits be lowered. This conflict caused repercussions all over Washington. The Joint Atomic Energy Committee set up hearings on the issue which were designed to be the most thorough yet. Columnists and newsletter publishers vied with one another to get the international report, which had been published in England, but which was not available here. A Washington science writer, criticized for one of his stories on the issue, defended himself before the Congress. The controversy even reached the confirmation hearings of former AEC chairman Strauss. One commentator suggestively pointed to the fact that the National Committee on Radiation Protection and Measurement has four members who are AEC employees.

United States Report

In its report, the National Committee on Radiation Protection and Measurement doubled its estimate of the amount of strontium-90 that could be allowed to accumulate in the human body without

causing an "unacceptable" hazard. It also increased by one-quarter the maximum permissible concentrations of the radioactive material in water, food, and milk.

The committee, the nation's highest advisory group on radiation protection, made its recommendations in the new issue of *Standards for Protection Against Radiation,* which was published last month. The new handbook is the result of 5 years of work by a subgroup of the national committee headed by K. Z. Morgan of the Oak Ridge National Laboratory. It is designed primarily to establish the amounts of radioactive materials that may be permitted to enter the bodies of workers at atomic energy installations and of persons living near such sites.

International Report

The organization which issued the conflicting report is the International Commission on Radiological Protection. This group, which was founded in 1928, has 13 members from seven countries. The chairman is R. M. Sievert of Sweden. The U.S. representative is Lauriston S. Taylor, who is also chairman of the National Committee on Radiation Protection and Measurement. In its recommendations, which were formulated 9 September 1958 but only recently released, the commission advised a further lowering of the "permissible" limits of radiation. If the international group's standards were adopted in this country, the current permissibility limits for industrial workers would be reduced to as low as one-tenth their present level. The estimated tolerable level of radiation from fallout would also be reduced, by about one-third. The basic criterion behind these standards and those of the national group is the amount of radiation the human body can receive without causing an "unacceptable" hazard.

Discrepancy Cited by Critics

Immediately after the international report became available, criticism of the national report began to mount. The discrepancy between the two reports was cited in conjunction with the suggested possibility that the United States, with its heavy investment in atomic energy, was presenting a misleading view of the dangers involved. Adding to the confusion were other reports which have appeared since publication of the U.S. committee's report. One that received wide comment was that of the General Advisory Committee of the Atomic Energy Commission. In general, this report lauded the commission for its work to date and stated that all "significant" data on radiation hazards had been made available to the public. It also gave a brief review of recent developments and an evaluation of radiation dangers. This report was criticized almost immediately by individuals outside the AEC. Many commentators pointed to the fact that there were no scientists trained as geneticists on the advisory committee. Thus, the question of possible genetic hazards over the years as opposed to physiological dangers in the present and in the near future remains unanswered. Criticism of the report reached a peak when Ralph Lapp, physicist and writer, described it as "shocking" and called for a presidential committee of inquiry to examine it.

Congressional Action

In the Congress, the special subcommittee on radiation of the Joint Atomic Energy Committee held 4-day hearings on the dangers from fallout. Testimony given before the subgroup tended to support the view that radiation dangers have been inadequately assessed. Witnesses said that, as a result of atomic weapon tests last fall, there is a record amount of radioactive debris in the stratosphere. This debris, they stated, can be expected to fall very rapidly and, because of the structure of the atmosphere, to fall principally on the Northern Hemisphere. As a result, the committee was told, radioactive fallout can be expected to double in the next few years. These reports drew expressions of concern from the subcommittee chairman, Chet Holifield (D-Cal.). He pointed to the fact that the amount of radioactive debris being created was far in excess of a safety limit of 10,000 kilotons annually, which was recommended by scientists during the subcommittee's fallout hearings in 1957.

Eisenhower Speech Highlights Basic Research Symposium

A 3-day symposium on basic research drew more than 225 of the nation's top scientists, educators, and industrialists to New York's Rockefeller Institute on 14–16 May. Although no summary or list of resolutions was issued at the end of the meeting, there was general agreement on a number of points. One was that basic scientific research is part of the general scholastic effort of the country and that any actions or attitudes that advance that general effort help further basic research. The need for means of support for research in addition to the individual project grant was also frequently cited by speakers and panelists. A third point on which there was general agreement concerned the desirability of having the research worker teach and the teacher do research for short periods during their careers.

The meeting, held under the joint auspices of the National Academy of Sciences, the AAAS, and the Alfred P. Sloan Foundation, provided a forum at which the conferees set forth and examined the facts concerning the support of basic scientific research in the United States. Governmental support was one of the main topics before the symposium, and the major news event of the meeting was President Eisenhower's announcement, at a dinner for the scientists, that he would ask Congress for $100 million for a new linear high-energy accelerator. The device, to be built at Stanford University in California, will be 50 times longer than the largest accelerator of its kind known to be in operation today.

Papers and Discussions

A total of 12 basic papers were read at the symposium, which was held under the great blue hemisphere of the institute's Caspary Hall. Robert Oppenheimer spoke first, on the "Importance of New Knowledge." He was followed by Alan T. Waterman of the National Science Foundation and William O. Baker of Bell Telephone Laboratories, who spoke, respectively, on "Basic Research in the United States" and the "Paradox of Choice"—an examination of management's role relative to an industrial laboratory's research. A discussion period followed these three presentations. Questions from the audience were answered by the speakers, who were joined by three or four other panelists. This practice was followed in each of the other three sessions, at each of which three papers were given. These sessions were concerned with basic research in various types of educational institutions, with basic research in various types of laboratories, and with financial support of basic research by government, industry, and private philanthropic organizations.

Eisenhower's Speech

The scientists attending the conference were joined by about an equal number of representatives from industry, from the fields of publishing and education, and from other professions for a dinner at the Waldorf-Astoria. Detlev W. Bronk, of the National Academy of Sciences and the Rockefeller Institute, acted as host during the dinner and introduced the speakers. Brief talks were given by James Killian of the Federal Council for Science and Technology and by Crawford Greenwalt of Dupont. The President then spoke, offering a brief review of the relationship between government and science, and announcing the proposal for the accelerator.

The papers and précis of the discussions at the New York meeting will be published by the AAAS, probably in the

fall. Dael Wolfle, executive officer of the association, will be the editor. Mark H. Ingraham, dean of the College of Letters and Science at the University of Wisconsin, was originally designated editor, but illness prevented his attendance at the conference. The volume, which is now in preparation, is expected to give wider distribution to the deliberations and suggestions of the conferees.

U.N. Space Group Establishes Scientific and Legal Committees

The United Nations Ad Hoc Committee on the Peaceful Uses of Outer Space convened for its first meeting on 6 May. The committee consists of 18 member states: Argentina, Australia, Belgium, Brazil, Canada, Czechoslovakia, France, India, Iran, Italy, Japan, Mexico, Poland, Sweden, the U.S.S.R., the United Arab Republic, the United Kingdom, and the United States. However, the delegations of Czechoslovakia, India, Poland, the United Arab Republic, and the U.S.S.R. did not attend the meeting. These countries have indicated that they would not participate in the work of the Outer Space Committee because they feel that its membership is unbalanced in favor of the West.

The committee is to report to the next session of the General Assembly on the following: (i) the activities and resources of the United Nations, of its specialized agencies, and of other international bodies relating to the peaceful uses of outer space; (ii) the area of international cooperation and programs in the peaceful uses of outer space that could appropriately be undertaken under United Nations auspices for the benefit of states, irrespective of the status of their economic or scientific development; (iii) future organizational arrangements to facilitate international cooperation in this field within the framework of the United Nations; and (iv) the nature of the legal problems that may arise in carrying out programs to explore outer space.

Subcommittees Established

At its opening session, the committee decided without objection and without a vote to form two subcommittees, one on the scientific aspects of the problem, the other on the legal issues involved. The establishment of the two subcommittees was proposed by Henry Cabot Lodge of the United States. The subcommittees will start work on 26 May and are expected to submit reports to the main committee by the middle of June. The *ad hoc* committee will consider the reports and then start drafting its statement for the forthcoming session of the General Assembly. It was agreed that the latter document should be completed by the end of July at the latest.

The committee also decided to request the Secretary General to report to the committee at an early date on the activities and resources of the United Nations, of its specialized agencies, and of other international bodies relating to the peaceful uses of outer space. It was agreed that consideration of another topic assigned to the committee by the General Assembly—future organizational arrangements—should be taken up by the committee only after submission of the reports of the two subcommittees and of the Secretary General.

Koto Matsudaira, permanent representative of Japan to the United Nations, was elected chairman of the *ad hoc* committee; Mario Amadeo, permanent representative of Argentina, vice chairman; and Joseph Nisot, permanent representative of Belgium, *rapporteur*. The United States has designated Hugh L. Dryden, deputy administrator of the National Aeronautics and Space Administration, as its representative on the scientific subcommittee, and Loftus E. Becker, legal adviser to the State Department, as its representative on the legal subcommittee. This country has prepared a series of documents on the topics to be studied by the committee.

U.S. Delegates Express Views

In an address in which he formally introduced a U.S. working paper, Dryden commented that the fields of application of satellites so far identified were those of meteorology and weather forecasting, long-distance communication, navigation, and geodetic measurements. Dryden felt that the three substantive areas which could most fruitfully be examined by the Scientific Subcommittee are space science, satellite application in other areas, and manned exploration of space. There is need for international cooperation in all three fields, he stressed. Joint efforts in the investigation of the ionosphere and the fundamentals of radio propagation through the upper atmosphere are required to obtain the world-wide coverage that alone can provide a complete picture, he added. "But most of all, space research needs to draw upon an entire world for its ideas," Dryden declared.

Among other things, Dryden mentioned that "in the near future" it would be possible to check the general theory of relativity by comparing the rate of a satellite-borne atomic clock with the rate of a similar clock on the ground. In the area of biosciences, he observed, "perhaps even more exciting is the possibility of finding life forms on other planets."

In the field of meteorology, Dryden said that satellites would open up the possibility of a world-wide system for observing the weather, with resulting benefits to agriculture, transportation, and other weather-dependent activities throughout the world.

A communications satellite, he said, might well lead "to vastly improved world-wide communications in terms of speed capacity, reliability and possible economy." Further, a navigational satellite might provide the basis for an all-

Henry Cabot Lodge (United States), Hugh L. Dryden (United States), and Sir Pierson Dixon (United Kingdom) converse after the opening session of the first meeting of the United Nations Ad Hoc Committee on the Peaceful Uses of Outer Space. [United Nations]

weather, long-range navigational system for surface vehicles and aircraft.

Loftus E. Becker also addressed the committee. In describing "the initial thinking" of this government on the program for the subcommittee on legal problems of outer space, he stressed that it was "desirable to make explicit the essential understanding that the application of the U.N. Charter and the Statute of the International Court of Justice is not limited to the confines of the earth; these instruments are applicable to the relations of earthly states in outer space as well." He pointed out that the mandate given by the General Assembly to the committee was that "of constructing a rationally ordered framework within which are posed a series of questions calling for legal examination and investigation." The committee was thus not called upon to formulate immediate answers to these questions or to "study in depth" these legal questions with a view to proposing definite rules.

An "ordered catalogue of necessary legal questions," not the determination of substantive rules, was the task before the Legal Subcommittee, in his view. An effort to agree now on any comprehensive code, he believed, might "come to naught, yield a small set of maxims of extreme generality, or produce an unworkable regime which would be dangerous in its giving of a temporary illusion of certainty."

Most of the representatives at the meeting made a special point of mentioning their regret at the absence of the five delegations that had boycotted the session and expressed the hope that they would reconsider their decision. As leader of this group, the U.S.S.R. has said that the boycott will continue until the committee is reorganized so that the total number of Soviet-bloc and neutralist delegates equals the number of Western members. No definite action on any aspect of the outer space problem can be taken without the agreement of the nations that lead in space research.

Nuclear Development Agency

A compact providing for the establishment of a nuclear development agency to operate in 15 southern states of the U.S. has been approved by representatives of the states involved. Under the pact, an interstate agency would promote greater use of atomic energy in the South's industry, science, and agriculture.

The agency, which was proposed last October at the Southern Governors Conference, may eventually operate its own research installation for the benefit of the member states. In addition to the general promotional aim, the proposed agency would collect and disseminate information about civilian uses of atomic energy, conduct training programs, study health and safety standards, and act as a licensee of the federal government in the matter of conducting research activity.

The pact now goes to the conference's nuclear committee. It must also be approved by Congress and by the legislatures of the participating states. These are Alabama, Arkansas, Florida, Georgia, Kentucky, Louisiana, Maryland, Mississippi, North Carolina, Oklahoma, South Carolina, Tennessee, Texas, Virginia, and West Virginia.

Rockefeller Institute Establishes $10,000 Foreign Fellowships

The Rockefeller Institute has announced that four distinguished research fellowships will be awarded each year to young scientists in England, France, Denmark, and Sweden for study and investigation at the institute. The fellows will be appointed by the Royal Society of London, the French Academy of Sciences, the Royal Danish Academy of Sciences and Letters, and the Swedish Royal Academy of Sciences. Each fellow, who will carry the designation of his sponsoring society, will receive an annual stipend of $10,000, with an additional $1000 for travel in this country.

The new fellowships will be supported by income from a bequest to the institute of approximately $1 million from the estate of the late Sophie D. Fricke of New York, who died on 1 March 1958. Miss Fricke was born in Jersey City, but lived most of her life in New York, where she was confidential secretary to many prominent business executives. Through wise investment of her personal savings she amassed a fortune, which she left for the furtherance of human welfare through support of science. The trustees of the institute have authorized use of the income from the Fricke fund for the triple purposes of fostering international understanding, training scientists of exceptional promise, and supporting significant research. The first Sophie Fricke fellows appointed by the foreign academies will begin their work at the institute this coming autumn.

Program on Weather Modification

A program of research in weather modification was announced last month by the National Science Foundation. It consists of 13 grants for laboratory research, field experiments, evaluation of present theory and practices, and conferences on modern meteorological methods directed toward weather modification. The program has the objective of studying more intensively than has been attempted before the scientific basis of weather modification, through support of competent scientists working in cloud physics, atmospheric physics, and allied fields.

In the laboratory, freezing nuclei will be examined with the electron microscope to determine their nature and make-up. Tests will be made to find the most efficient freezing nuclei. In the field, numerous cloud-seeding experiments are planned, in which silver iodide and other agents will be used to find out more about how clouds form and grow, about the precipitation mechanism, and about variations in precipitation that may be attributed to cloud-seeding. Other means of modifying clouds and weather will be studied, including introduction of layers of lampblack and other heat-absorbing agents to change artificially the radiation balance of clouds, and inducing local changes in atmospheric electricity with probable resultant changes in the growth of cloud droplets and precipitation.

The program also includes study and improvement of the physical and statistical evaluation methods employed in determining the results of any seeding operation. Perhaps the greatest difficulty in this field is to differentiate clearly between man-caused rainfall and the rainfall that would have occurred if man had not intervened.

AAAS and Westinghouse Form Awards Managing Committee

The composition of the Science Writing Awards Managing Committee for the new AAAS–Westinghouse Science Writing Awards is now complete. The Westinghouse Electric Corporation has named Charles N. Fry, director of public relations, and Harry R. Gail, manager of research and development information in the public relations department, as its members; the National Association of Science Writers has named Jules Billard, an associate editor of *U.S. News and World Report,* and Nate Haseltine, science writer for the *Washington Post and Times Herald;* and the American Association for the Advancement of Science has named its executive officer, Dael Wolfle, and Hans Nussbaum, business manager.

In a meeting held on 3 March, it was also agreed that the AAAS would designate an administrator of the award competition, who will also serve as chairman of the managing committee. Graham DuShane, editor of *Science,* has agreed to accept this responsibility.

News Briefs

The National Education Association has reported that the nation's teacher shortage is easing somewhat in science, mathematics, and foreign languages. Although the association's annual survey indicates that the over-all teacher shortage will be at the same level this year as it was last, there will be increases in the number of science, mathematics, and foreign language teachers graduating in the college classes of 1959. The number of new science teachers to be graduated will be 6984, an increase of 28 percent over 1958. There will be 4732 new mathematics teachers and 2005 new language instructors, increases of 37 and 23 percent, respectively, over last year's totals.

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The Wisconsin Sociological Association was founded this month by representatives of various educational institutions in the state. Plans were made for future activities, including a society publication and a meeting in the fall of this year. About 30 Wisconsin sociologists attended the founding session.

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Under a new plan introduced by the superintendent of schools, New York City teachers would receive salary increases ranging from $280 to $510. The measure, which will be considered by the city Board of Education in late May, would increase the basic starting pay of $4000 a year by $500 and the present maximum of $7600 by $300. The largest increase—$1000—would go to nine associate superintendents of schools.

The proposal was made 6 May by John J. Theobald, superintendent of schools, in a broadcast over the school system's FM radio station. If approved, the new schedules would take effect 1 September of this year. The plan will cost $18 million annually, and will benefit all of the city's 40,000 public school teachers.

* * *

The Michigan Nucleonic Society was established at a general meeting on 11 March at the University of Michigan. The society has been active for the last 2 years under the name of the Nucleonic Discussion Group. Membership is open to all interested in work with radioisotopes and radiation and allied fields. The board chairman is Luther Preuss. For information write to the secretary, Arthur Solari, 509 Linden St., Ann Arbor, Mich.

* * *

Papers presented at the Inter-American Symposium on the Peaceful Application of Nuclear Energy, the first general meeting on the peaceful uses of the atom in the Western Hemisphere, have been published by the U.S. Atomic Energy Commission and released for sale to the public by the Office of Technical Services, U.S. Department of Commerce. Representatives of 19 Latin countries and the United States participated in the symposium, which was held at Brookhaven National Laboratory in May 1957.

Grants, Fellowships, and Awards

Foreign study. About 900 Fulbright scholarships for graduate study or predoctoral research in 27 different countries will be available for the 1960–61 academic year. In addition to the Fulbright awards, scholarships for study in Latin America under the Inter-American Cultural Convention are also offered for 1960–61. The Institute of International Education administers both of these programs for the U.S. Department of State.

The Fulbright scholarships cover travel, tuition, books, and maintenance for one academic year. Countries participating in the program include Argentina, Australia, Austria, Belgium and Luxembourg, Brazil, Burma, Chile, China, Denmark, Ecuador, Finland, France, Germany, Greece, Iceland, India, Iran, Italy, Japan, Netherlands, New Zealand, Norway, Peru, Philippines, Spain, Turkey, and the United Kingdom. Awards for study in Eire are also available under an arrangement similar to the Fulbright program.

The IACC program makes one or more awards available for graduate study in the following Latin American countries: Bolivia, Brazil, Chile, Colombia, Costa Rica, Cuba, Dominican Republic, Ecuador, Guatemala, Haiti, Honduras, Mexico, Nicaragua, Panama, Paraguay, Peru, and Venezuela. IACC scholarships cover transportation, tuition and partial to full maintenance.

General eligibility requirements for both categories of awards are (i) U.S. citizenship at time of application, (ii) a bachelor's degree or its equivalent, (iii) knowledge of the language of the host country sufficient to carry out the proposed study project and to communicate with the people of the country, and (iv) good health. A good academic record and demonstrated capacity for independent study are also necessary. Preference is given to applicants under 35 years of age who have not previously lived or studied abroad.

Applicants will be required to submit a plan of proposed study that can be carried out profitably within the year abroad. Those who plan to take dependents may be asked to submit a statement of their financial ability to provide for their round-trip transportation and maintenance.

Applications for Fulbright and IACC scholarships for 1960–61 will be accepted *until 1 November*. Requests for applications must be postmarked *before 15 October*. Those interested who are now enrolled students at a college or university should consult their campus Fulbright advisers. Others may write to the Information and Counseling Division, Institute of International Education, 1 E. 67th St., New York 21, N.Y. or to any of IIE's regional offices.

The IIE also administers 200 fellowships for graduate study abroad that are offered by foreign governments and universities in 15 countries. Applications for the 1960–61 fellowships will be accepted *until 1 November*.

The scholarships cover tuition and varying amounts of maintenance in universities in Austria, Denmark, France, Germany, Iran, Israel, Italy, the Netherlands, Sweden, Switzerland, Canada, Brazil, and Mexico. Students applying for Italian university awards or Austrian, Danish, French, German, or Netherlands government awards may apply for a Fulbright travel grant to supplement their scholarships. Two additional awards, offered by an American foundation are for any country in the Far East, South or Southeast Asia, and Africa.

General eligibility requirements are United States citizenship, a bachelor's degree or its equivalent before departure, language ability sufficient to carry on the proposed study, and good health. Preference is given to applicants under 35 years of age who have not had extensive foreign experience. While married persons are eligible for most of these described awards, the stipends are geared to the needs of single grantees.

Neurophysiology. The Dysautonomia Association has announced that funds are now available for basic or clinical research in neurophysiology. The association will support research related to the clinical entity known as familial dysautonomia, a disease of the autonomic nervous system. Support will also be provided for any type of study of nervous-system functions that would contribute to understanding of a disease process. For information, write to Harold M. Newman, President, Dysautonomia Association, 2 W. 46 St., New York 19, N.Y.

Scientists in the News

EUGENE P. WIGNER, Thomas D. Jones professor of mathematical physics at Princeton University, recently delivered the first Richard Courant lecture at New York University. His subject was "The Unreasonable Effectiveness of Mathematics in the Natural Sciences." The Courant lectureship in mathematical sciences was established in January 1958 to honor the former scientific director of New York University's Insti-

tute of Mathematical Sciences. Courant, who came to New York University in 1934, retired last year. As professor emeritus of mathematics he is now science adviser to the university.

JOSHUA LEDERBERG, head of the department of genetics at Stanford University School of Medicine and cowinner of the Nobel Prize in 1958, left in May for a 1-month trip to Europe. Mrs. Lederberg, who is also a geneticist and works with her husband, is accompanying him. On 29 May, Lederberg will deliver his Nobel Prize lecture on genetic recombination at the Royal Caroline Institute of Medicine in Stockholm, Sweden.

EDWIN B. WILSON, eminent mathematical physicist and senior scientific adviser, was recently honored at an 80th-birthday celebration, sponsored by the American Academy of Arts and Sciences, in Brookline, Mass. He has served as president of the academy and in many other capacities for nearly a half century.

NORMAN A. HASKELL, scientist at the Geophysics Research Directorate, Air Force Cambridge Research Center, Bedford, Mass., will deliver the Guenter Loeser memorial lecture on 26 May in Boston, Mass. His subject will be "The Detection of Nuclear Explosions by Seismic Means."

S. S. VISHER, professor of geography at Indiana University, has been awarded the Outstanding Achievement Award of the Association of American Geographers.

JOHN C. HOUCK, director of the Surgical Research Laboratory, Georgetown University, Washington, D.C., will become director of the Biochemical Research Laboratory, Children's Hospital Research Foundation, Washington, D.C., effective 1 July.

DONALD J. MONTGOMERY, professor of physics at Michigan State University, will lecture and conduct research at the University of Grenoble, France, for a year, beginning in September.

PHILIPPE LECORBEILLER, professor of general education and applied physics at Harvard University, will retire 30 June and become professor emeritus. A specialist in the electronics of communication, electromechanical systems, and acoustics, LeCorbeiller came from France to the United States in 1941. He taught electronics to Army and Navy personnel at Harvard during World War II and joined the Harvard faculty in 1945 as lecturer in applied physics, becoming a professor in 1949. He was formerly professor of the theory of electricity in the Graduate School of Communication at the University of Paris, and senior assistant to the professor of calculus at the Ecole Polytechnique. He will remain in Cambridge to complete a book on the role of science in society.

Scientific visitors to the United States from the United Kingdom are as follows:

H. H. PEARCEY, senior principal scientific officer, Aerodynamics Division, National Physical Laboratory, Teddington, will be in this country 11 June–4 July. He will attend the national summer meeting of the Institute of Aeronautical Sciences in Los Angeles, 16–19 June. (In Washington, 11–13 June.)

T. S. WORK, member of the Medical Research Council's scientific staff at the National Institute for Medical Research, London, arrived 16 May from Jamaica. He will take part in a symposium on protein synthesis at the University of Wisconsin, 10–12 June. Itinerary: New York; Washington (23–27 May); New Haven, Conn.; Boston; Cleveland; Montreal, Canada.

ROGER NEWMAN, formerly with General Electric's research laboratories, has joined Hughes Aircraft Company as head of the physics department of the materials research laboratory of Hughes Products Group's semiconductor division; THOMAS D. HANSCOME, formerly chief scientist at the U.S. Navy Research Laboratory, and WALTER G. WADEY, formerly research physicist at Yale University, have joined the company's nuclear electronics department.

RAYMOND A. JENSEN, former chief of the abstract section, Office of Technical Services, U.S. Department of Commerce, has been appointed executive secretary of the National Federation of Science Abstracting and Indexing Services, Washington, D.C.

STACY R. GUILD, director of the anatomical and pathological research laboratory in the department of otolaryngology at Johns Hopkins University School of Medicine, will retire on 1 July and become professor emeritus. He will continue as lecturer in the department of environmental medicine in the School of Hygiene and Public Health. Guild, who was also associate professor in the Medical School, retires after 33 years of service at the university. He left the University of Michigan School of Medicine in 1926 to join Johns Hopkins.

Recent Deaths

LORENZ S. BAUR, Carcinoma, Ohio; 50; director of nutritional research at Ross Laboratories, Columbus, Ohio; had been with the company for 18 years; associate editor of *Pediatric Currents, Nursing Currents,* and *Currents in Hospital Administration*; 8 Apr.

HENRY R. BOYES, Detroit, 69; medical missionary and director of the Kennedy Memorial Hospital of the United Presbyterian Church in Tripoli, Lebanon; 1921–58; 9 May.

EDWARD T. BUTLER, Philadelphia, Pa.; 49; chemical engineer and director of research and development for the Broadway Maintenance Corporation, New York; 9 May.

WILLIAM CONE, Montreal Canada; 62; chief of the neurosurgery service at the Montreal Neurological Institute and professor of neurosurgery at McGill University; had been connected with the university since 1928; 4 May.

FRANK A. CRAIG, Philadelphia, Pa.; 82; a leader in tuberculosis control and professor emeritus of clinical medicine of the University of Pennsylvania since 1951; had been associated with the university for 50 years; staff member of the Henry Phipps Institute, Philadelphia, 1903–54; 11 May.

HARVEY W. CULP, Ossining, N.Y.; 55; psychology teacher and former dean at Briarcliff College; research executive for the Foundation of Integrated Education in New York and president of the Ossining Board of Education; former research chief for Babcock and Wilcox; 11 May.

CARROLL W. GRIFFIN, Poughkeepsie, N.Y.; 58; chairman of the Vassar College department of chemistry and a faculty member for 27 years; research associate at Rutgers University, 1931–32; author of sections in his field in the *Encyclopedia Americana*; 5 May.

PIERRE MASSON, Montreal, Canada; 79; tumor specialist and professor of pathological anatomy at the University of Montreal; member of the Royal Society of Canada; 11 May.

ARTHUR S. RANDAK, Old Greenwich, Conn.; 46; assistant manager of the eastern division of Sinclair Refining Company, New York; director of the Sinclair Research Laboratories and chairman of the refining company's motor fuels steering committee; 5 May.

HEINRICH F. WOLF, New York; 86; physician specializing in physical medicine; director and founder of the Eastern School for Physician's Aides, a training center for laboratory and x-ray technicians; former chief of the department of physical medicine at Mount Sinai Hospital; author of the textbook *Physical Therapy*; 12 May.

Book Reviews

Evolution, Marxian Biology, and the Social Scene. Conway Zirkle. University of Pennsylvania Press, Philadelphia, 1959. 527 pp. $7.50.

On 14 December 1958 *Pravda* denounced the author (Conway Zirkle) and the reviewer (Theodosius Dobzhansky) of this book as "reactionaries," because of their opposition to Lysenko's perversion of science. However, the two "fellow-reactionaries" find themselves at odds about several matters that are discussed in this book. The purpose of the book is to document the author's belief that ". . . a Marxian biology exists as a destructive, threatening, and well-organized cult. . . . Once we have traced its historical development we can show how it has modified the growth and development of our own beliefs and how it has pervaded our own thinking. Today, it is imbedded not only in the writings of socialist theoreticians and left-wing philosophers but in fields and disciplines that, ostensibly, are not marxian at all. It has contributed to our present ideologies much more than appears on the surface." The author is on the track of Marxian biology throughout the 12 chapters of his book. About half of the chapters present concisely the history and the modern state of genetic and evolutionary theories, and the other half are concerned mainly with the social and political implications of these theories. (These latter chapters are: "The beginnings of Marxian biology"; "The impact of evolution on society and on the social studies"; "Marxian biology and beautiful letters"; "Marxian biology in the communist world"; "Marxian biology and sociology"; and "Epilogue"). Yet, I find myself unsure about just what constitutes Marxian biology. On pages 85 and 112 it is stated that Marx and Engels (i) accepted organic evolution, (ii) accepted natural selection in place of teleology, (iii) rejected the Malthusian principle as the selecting agent, (iv) were disrespectful to Malthus as a person, (v) believed in the inheritance of acquired characters, and (vi) stressed the role of the environment as a cause of the variability of the human species. At present, "The attitude assumed by the Marxians toward eugenics is one of the best identifications we have of their peculiar biology, in both its overt and privy forms" (page 142). I question the diagnostic value of these identifications. Darwin, who was not a Marxian biologist, accepted points 1, 2, and 5, and was not very sure about 6. Because he published in 1946 an excellent history of the idea of the inheritance of acquired characters, nobody is better qualified than Zirkle to know that, before the advent of modern genetics, inheritance of acquired characters was an admissible working hypothesis, and that at present the belief in such inheritance is an identification not so much of Marxism as of simple ignorance of elementary biology.

As to skepticism concerning eugenics, is this really diagnostic of Marxism, even "when combined with the other symptoms of the Marxian syndrome"? The author admits (page 166 and elsewhere) that the excesses of social Darwinism and of some brands of "race-and-class" eugenics have produced a perhaps not wholly illegitimate recoil reaction among both biologists and sociologists, and that this revulsion has harmed also the scientific study of the role of the biological factors in human affairs. The more diffuse the characteristics of a syndrome, obviously the more pervasive will its elements be found to be, and this will result in some perplexing situations. The author himself notes with embarrassment (page 304) that "To include Alfred Lord Tennyson among the writers who helped to spread Marxian biology might seem to be the height of something or other." Other "heights" are the inclusions among the "helpers" of such diverse figures as Samuel Butler, G. B. Shaw, Alphonse Daudet, Jack London, E. Bellamy, Lester Ward, H. G. Wells, P. Kropotkin, O. Hertwig, and even Rudyard Kipling! I am proud to qualify for membership (probably of a privy kind) in so distinguished a company (page 493). The penetration of Marxism in our culture would seem to be wide indeed, as the author claims. But I respectfully suggest that, in the West, Marxian biologists are not hidden under every laboratory bench; that they are a rare species; and that the Lysenko scandal has reduced their ranks to near-extinction.

The situation in the Soviet Union is, of course, a different story. When, in the fullness of time, the amount of harm done by the Michurinist-Lysenkoist incompetents to Russian cultural and economic life will be estimated, a sizable fraction will probably be found in that they caused a whole generation to be miseducated on prescientific superstition masquerading as biology. This sad tale has been told many times, and a concise summary is included in the book under review. A puzzle which remains unsolved is why the rulers of U.S.S.R. have committed a blunder so egregious. It may be that a blunder is a blunder, and irrational behavior cannot be justified rationally. The author has a quite different view, which is perhaps the ideological axis of his book. He believes that "Environmentalism is so important to the communists that they would preserve it at all costs, even if it meant the suppression of scientific discoveries and the destruction of research institutions." I dissent. Marxism is just as compatible with the *fin de siècle* brand of Lamarckian environmentalism as it is with modern scientific biology. True, Marx, Engels, Lenin, Timiriazev, and other divinities of the communist pantheon believed in the former. But is this not what one expects those familiar with only the *fin de siècle* biology to believe in? On the other hand, there were a few geneticists in the West, and among them even very good ones, who were also communists. They looked very foolish indeed when trying to invent excuses for Lysenko's debaucheries, but there is no evidence that they found it difficult to square their political views with scientific biology. The author does not know, or at least does not mention in his book, that in the twenties it was apparently a touch-and-go proposition whether genetics or hotchpotch Lamarckism would be declared to be the Party Line. And why not? The author himself points out that Lamarckism may lead to some conclusions outrageous to a thinking Marxist (page 333 and elsewhere). The historical antecedents of the destruction of genetics in Russia are yet to be fully clarified.

I consider it a serious error to claim that the sets of political beliefs known as Marxist, Leftist, and even liberal, are compatible with some "Marxian biology" and not with modern biology, genetics, or evolution theory. This comes dangerously close to a contention that a different set of political views follows from modern biology. Lysenko and his adherents declared genetics to be a bourgeois, reactionary, idealistic (and so forth) science; is the non-Marxian biol-

ogy a Tory biology, or an Old-Guard-Republican biology? I do not believe this to be the case; but those who might wish to contend that it is will find in the book under review enough material to support these contentions.

THEODOSIUS DOBZHANSKY
Department of Zoology,
Columbia University

Between Earth and Space. Clyde Orr, Jr. Macmillan, New York, 1959. ix + 253 pp. Illus. $4.95.

This book is a fine contribution to the lengthening shelf of popular science volumes. It deals with the thin atmospheric envelope around the earth. The origin, physics, and chemistry, of our air mantle are well presented by the author, who wields a very facile pen. With a sense of the dramatic he introduces the reader to the realm of unending storms, large and small. The beautiful aspects of clouds, auroras, and the many halo phenomena are deftly sketched and the causes are well explained.

As a chemical engineer, Orr is particularly at home in matters dealing with air suspensions and atmospheric pollution. The past and present state of affairs and the anticipated future problems in the struggle for clean air concern people in all walks of life. Here is a thought-provoking summary of the issue we face in this field.

It is a pity that such a well-written book is marred by a number of small slips. Some of them are probably attributable to the author's acceptance of news stories as sources. Among these slips are erroneous reports of a wind of 392 miles per hour and of a tornado with translatory speed of 130 miles per hour, and confusion of the maximum wind speed recorded at Mount Washington, in 1934, with wind speed during the New England hurricane of 1938. One must also raise an eyebrow at the reference to thunderstorms "over land" at the North Pole, and at the labeling of the upwelling cold water along the West Coast as "an Arctic current." The professional meteorologist will also find, here and there, too ready an acceptance of solar influences which still have to be proved.

These inaccuracies do not greatly detract from the general merits of the book. Most of them could be readily remedied in a second printing. They are well compensated for by the technically acceptable discussions on climate, on the behavior of the weather, on the difficulties of forecasting, and on the possibilities of modifying the weather. The section on upper atmospheric exploration by rockets and satellites is as up-to-date as one can hope for during a period of explosive development. Eight good plates accompany the text, but a few more illustrations might have helped the reader to visualize better many of the phenomena.

The book has a good index and a useful reading list of titles of over 200 books and articles. Many of these publications provided raw material for the author. I am sure that most of the original writers of the popular or semipopular material cited would feel gratified at the effective use which has been made of their contributions. Anyone who wants a quick and easy glimpse at developments in atmospheric science can get it from this book. It is also well suited for high-school science libraries.

H. E. LANDSBERG
Office of Climatology,
U.S. Weather Bureau

Nomograms for Chemical Engineers. Om P. Kharbanda. Academic Press, New York, 1958. xi + 247 pp. Illus. $15.

The book consists of a compilation from the literature of over 100 nomograms, including many devised by the author. Nomograms of particular interest to the chemist and chemical engineer for the determination of physical properties and the solution of problems on unit operations are presented. Each nomogram is accompanied by descriptive material, including the theoretical and empirical basis of the nomogram, literature references, and an illustrative example.

The format of the book is particularly convenient; a full 8½- by 11-inch page is devoted to each nomogram in most cases. The main drawback of such a compilation is the necessarily restricted selection of nomograms; also, the data available for the construction of each nomogram are often incomplete; for example, only selected substances are included among the physical properties.

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Photomicrography. Roy M. Allen. Van Nostrand, New York, ed. 2, 1958. xiii × 441 pp. Illus. $9.

Relatively few changes have been made for this new edition. The basic methods of an expert in black-and-white photography are again presented. New pictures illustrate the chapter on modern photographic equipment, and the comments are the equivalent of a personal shopping tour with the author. For many readers this chapter will justify publication of the book. A new chapter gives a rambling account of phase microscopy and mention of interference microscopy, the latter section being interrupted for an account of a variable-phase microscope. In neither section are the problems of photography considered. The chapter on the electron microscope (five pages in the first edition) now occupies 12 pages.

The chapters on fundamental principles, homemade equipment, photographic processes and equipment, and microphotography remain about the same, except for the addition of some illustrations and descriptions of some new equipment. The chapter on microphotography is so meager and out of date that it might well have been omitted. In the chapter on techniques and processes some information on modern equipment and methods has been included, but there should have been more pruning of material on obsolete, discontinued equipment. None of the stronger sources for fluorescence microscopy are mentioned, and statements such as "only low powers can be used, as otherwise exposure times may extend into many hours" should have been dropped in this edition. In discussing stereoscopic photomicrography the author makes no mention of the use of twin-lens cameras, although the method has been used in several places for several years.

The most serious omission occurs in the discussion of modern color photomicrography. Although the ancient Lumière and Findlay plates are still described, there is no mention of electronic flash techniques or of the modern equipment and techniques for controlled time-lapse cinephotomicrography. Some photographic materials of one company are discussed, but the popular and useful Panatomic X film produced by this company is not mentioned. Only a few references are given—to Köhler's paper, a book by Morgan and Lester, an article in the *Scientific American* on microwriting, a book on microrecording, and publications of a photographic company. A few leading references to modern work would have been helpful and would have compensated, in part, for the omission of methods developed in the last few years.

Four new plates, on cast iron, steel, sago starch, and soluble coffee, have been added to the useful section illustrating good photomicrography and Allen's methods of achieving it. A comparison of the two editions reveals that the plates now show the wear of several printings.

Allen's is a personal book. Many of the methods described are basic to good

black-and-white photography and hence are just as useful today as they were yesterday. Allen's personal comments are apt and reflect years of experience. My review is intended to indicate the scope and place of the book and not to criticize an elder statesman of microscopy.

OSCAR W. RICHARDS
Research Center,
American Optical Company

Historical Geography of the North Carolina Outer Banks. Gary S. Dunbar. Louisiana State University Press, Baton Rouge, 1959. xii + 234 pp. Illus.

This well-written, logically organized, well-documented book deals with the barrier island chain between North Carolina's Cape Lookout and the Virginia line. These barrier islands lie far from the mainland. Since so much of the Banks consists of sandy waste, the reader constantly asks himself what it was that attracted the original settlers.

Though Roanoke Island is not a part of the Outer Banks, it is included in the study because of its proximity, cultural similarity, and historical significance. It was selected by the English settlers under Raleigh as the site for a colony and as a base from which to launch raids on the Spanish Indies; but, as the author brings out, the English "could not have made a worse selection." This colony became the "lost colony"; what happened to it is not known, but destruction by Indians seems probable. The colony contributed nothing to the permanent settlement of the Banks. The Jamestown colony of 1607, however, was able to start with a somewhat better knowledge of the topography and natural resources as a result of the Roanoke experiments.

It is primarily to describe the nature of the settlements of the Carolina Banks that this study was made and that the volume was published. An interesting and valuable part of the study deals with the introduction of plants by the settlers, who envisioned them as profitable export products; the new settlements positively had to produce some item or items needed by the mother country. Cultivation of mulberry trees for a silk industry, viniferous grapes, figs, lemons, almonds, olives, and cassava was tried; most of these projects either failed completely or showed little promise of success. By this time, however, the Virginia agriculturists had found in tobacco the economically successful crop they sought.

The author points out the value to the white settlers of the cultivated Indian plants—in particular of the great "crop trilogy," maize, beans, and cucurbits. Indian stores of corn were invaluable in helping the colonists through the first winters.

The first permanent settlement on the Outer Banks was made in the 1660's; almost all of these settlers were Virginians, who by this time had solved most of the problems of pioneering in the New World and who brought with them the Virginia system of growing tobacco on riverine plantations and of rearing livestock on necks and islands. The first homes on the Outer Banks were all built on the "hammocks" (variant of "hummocks")—wooded tracts usually slightly above the surrounding area and on the sound side. These "hammocks" are also the homesites today. Yards and gardens were enclosed by fences to protect them from roving livestock.

Fishing in the sound waters soon became an important activity. Menhaden fishing was unsuccessful, however, because the water in the sounds is so shallow that fish do not congregate there in great numbers and purse seines cannot be used effectively. North Carolina's impressive menhaden-fishing industry is carried on in outside waters and hence is of no direct concern to the "Bankers."

The tourist business seems to hold about the only promise for the "Bankers" today. A flood of tourists descends on the Banks each summer, raising the incomes of all local residents.

Extensive and detailed notes cover each chapter in this book; these are so interesting and so informative that even the casual reader finds himself delving into them. The person much interested in the area would find these notes one of the most satisfactory parts of the volume. The cartographic work is of high quality—accurate, meaningful, and pleasing to the eye. There is an excellent bibliography and a helpful index.

C. LANGDON WHITE
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The Measurement of Values. L. L. Thurstone. University of Chicago Press, Chicago, 1959. viii + 322 pp. $7.50.

This volume brings together 27 of Thurstone's hitherto scattered papers on the measurement of attitudes and subjective values. Included are all of his classic contributions to psychophysics.

When Thurstone went to the University of Chicago in the 1920's he began a sweeping reanalysis of the logic of psychophysics, the field of inquiry started by E. H. Weber and G. T. Fechner in an attempt to develop rigorous statements of the relations between sensations and the stimuli that produced them. One of Thurstone's distinctive contributions was to develop experimental methods and a rationale for dealing with values, attitudes, and similar subjective variables that cannot be related to physical quantities.

Thurstone's papers on subjective measurement and attitude measurement pretty completely made over the field of psychophysics, replacing its former limitations with a wide range of useful applications in the social sciences and substituting a systematic and meaningful understanding for the empirical "Weber's law" and "Fechner's law." Such papers as "A law of comparative judgment," "A mental unit of measurement," "Rank order as a psychophysical method," "The indifference function," "Theory of attitude measurement," and "The measurement of change in social attitude" are essential reading for any student of psychological measurement. But copies have been increasingly hard to acquire. The new book solves that problem and will be of great convenience.

The idea for the collection came originally from some of Thurstone's former students, but he himself was largely responsible for selecting the papers to be included. Mrs. Thurstone, always his close professional colleague, prepared an explanatory preface and saw the volume through the press.

DAEL WOLFLE
American Association for the Advancement of Science

Inside the Living Cell. Some secrets of life. J. A. V. Butler. Basic Books, New York, 1959. 174 pp. Illus. $3.50.

Perhaps the most useful way to evaluate this book is to compare it with R. W. Gerard's *Unresting Cells,* published almost 20 years earlier. Both books are popularizations of cell physiology, but popularizations at a very high level of sophistication. Each presents a view of the facts as seen through the prism of the author's own lucid and critical intellect. Both books are excellent.

It is interesting to note the large number of topics in Butler's book that were either entirely unknown in 1940 or but dimly foreshadowed: the existence and importance of deoxyribonucleic acid (DNA) and ribonucleic acid (RNA); heredity as a problem in code construction; the Watson-Crick model of DNA replication; the role of antivitamins; the relation of genes to enzymes, as shown by the Beadle-Tatum school; the strange behavior of bacteriophages; and Oparin-style theories of the origin of life. Who

could have foreseen such developments 20 years ago? (And what will be the living ideas in cytology 20 years hence?)

The only substantial adverse criticism that might be offered is of the photographic reproductions. The originals of these—electron micrographs of mitochondria, microsomes, viruses, chloroplasts, and muscle fibrils—are things of great beauty, but most of the beauty has unfortunately been lost in the reproduction. For the layman who is seeing these pictures for the first time, the plates will do, because he doesn't know what he is missing, but it is a pity they are not better. The author no doubt agrees with this criticism.

Toward the end of the book Butler branches out considerably from his stated topic to consider mind, instincts, memory, free will, ageing, death, and the purpose of it all. Admittedly, it may be possible some day to discuss many of these topics on a cytological basis, but that day still seems a long way off. However, since the author's discussions are short and often illuminating, one does not begrudge him these diversions. It is Tennyson's "flower in the crannied wall" again: it is only natural for the serious and thoughtful specialist to fancy he sees real connections between the tiny object to which he devotes his life and the cosmos itself. Sometimes he is right.

The author's approach is, throughout, a quantitative one; he emphasizes the importance of thinking in terms of exponential numbers and orders of magnitude. This approach may repel the layman who has become allergic to "math" (may his tribe decrease!), but it is certainly the best approach. At least physicists, chemists, and other scientists who are laymen in the field of cytology will find this a stimulating and informative work. It deserves to be as widely read as its great predecessor, *Unresting Cells.*

GARRETT HARDIN
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Communist Economic Strategy: The Role of East-Central Europe. The economics of competitive coexistence. Jan Wszelaki. National Planning Association, Washington, D.C., 1959. xii + 132 pp. $3.

This book, the first volume in the National Planning Association's series on the economics of competitive coexistence, is useful in assembling a variety of data on the trade of East-Central Europe with the Soviet Union and with the underdeveloped countries of the Far and Middle East. Its thesis is that East-Central Europe provides an industrial base which is important to the Soviet Union, both directly and in the latter's attempt to penetrate economically and politically into underdeveloped countries. At the same time it is admitted that the countries of East-Central Europe need raw materials and foodstuffs and that they are obliged to trade with the Soviet Union and tropical areas in order to acquire them.

The study is a short one, and it pays only limited attention to the evolving background of industrial and agricultural production in Poland, East Germany, Czechoslovakia, Hungary, Rumania, Bulgaria, and Albania, on which countries its interest is focused. It is not so rich in data, for example, as Nicholas Spulber's *The Economics of Communist Eastern Europe* (Wiley, New York, 1958). The major question it raises, however, is whether the purpose of this study, and of the series in which it is the first to appear, is analytical or pamphleteering. The task of an analytical study would be to determine how much of the expansion of Soviet and East-Central European trade (incidentally, the study does not use the words *expansion* or *increase* but always *trade drive* or *offensive*) makes no sense in economic terms, or has an economic cost and therefore can be regarded as political.

In the present instance, the National Planning Association's Special Project Committee starts out by asserting that "the recent policies of the Soviet bloc have been patently designed to secure influence and eventual domination over much of the uncommitted world" (page viii), and the author ends up by stating, "While the trade offensive of the East-Central European countries could perhaps be largely explained in economic terms, the aid drive undoubtedly rests on the political aspirations of the Soviet Union . . ." (page 113).

Many aspects of Soviet economic foreign policy, such as credits granted to underdeveloped countries and to the countries of East-Central Europe, are "patently" or "undoubtedly" uneconomic, just as United States aid to underdeveloped areas is "uneconomic" and could be described as penetration. But the expansion of foreign trade between the Soviet Union and Eastern Europe and between Eastern Europe and the countries of Asia must be described as economic, and not political, to the extent that it more closely follows the law of comparative advantage. The study does not directly pose the question of whether or not this is the case. The author asserts that trade is political; the reader is left with the uneasy feeling, however, that the facts indicate that the movement has been in the direction of more rather than less economic reason.

C. P. KINDLEBERGER
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New Books

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Reports

Regulation of Mitosis in Stentor coeruleus

Abstract. When *Stentor coeruleus* was cut into anterior and posterior halves, the micronuclei in the posterior half underwent mitosis about 5 to 6 hours later, as shown in stained preparations. It is suggested that the division of the micronuclei was initiated by metabolic changes which resulted from the lack of adoral membranelles.

Evidence is accumulating that a minimum amount of chemically bound energy is required for the initiation of mitosis. This is borne out by the investigations of Swann (*1*) and Bullough (*2*), who have demonstrated the need for a minimum energy supply for the initiation of mitosis in sea urchin eggs and mouse epidermis, respectively.

The role of energy supply in the rhythmic alternation between cell growth and cell division can be studied in the ciliate *Stentor coeruleus,* since its anterior end has a conspicuous energy-consuming ciliary apparatus, and because it contains a highly polyploid macronucleus, surrounded by a varying number of micronuclei, which extends throughout most of the cell. The organism, therefore, can be cut into halves in such a way that the two halves receive approximately equal shares of the macronucleus and the micronuclei, while only the anterior half receives the ciliary apparatus. The behavior of the nuclei in such halves can then be correlated with the energy consumption, which should be relatively increased in the anterior fragment but decreased in the posterior fragment, as compared with the energy consumption of the whole cell.

Schwartz found previously (*3*) that removal of the adoral apparatus in *S. coeruleus* results in a temporary contraction of the macronucleus similar to that seen during normal cell division. After regeneration of the organism, he found an unexpectedly large number of micronuclei—a finding which indicated that the micronuclei had undergone mitosis. Since this experiment has direct bearing upon the problem outlined above, we have repeated it in order to establish, in stained preparations, whether or not the loss of the adoral organelles is inevitably followed by mitosis of the micronuclei (*4*).

About 1 hour after division, a large number of cells were cut with a glass needle into anterior and posterior fragments, which received approximately equal parts of the macronucleus, together with micronuclei. At this stage, according to Weisz (*5*), the anterior and the posterior portions of the macronucleus are functionally equal. The living pieces were examined from time to time with the aid of a stereomicroscope. When left alone, both parts regenerated and, after more than 40 hours, divided. About 5 to 6 hours after the cutting, the macronuclei in the posterior fragments became contracted and remained so for more than 1 hour, while no contraction occurred in the anterior pieces. Those fragments whose macronuclei were contracted were fixed on albuminized cover slides (mercuric chloride-ethanol-glacial acetic acid), stained with alum carmine, and examined under a phase microscope. In 17 out of 125 cases the micronuclei were found to be in mitosis in the posterior fragments, whereas no mitosis and no increase in the number of micronuclei was observed in the anterior fragments, regardless of the respective sizes of macronuclei and fragments. In controls, the micronuclei did not undergo mitosis before the next cell division, which occurred about 24 to 30 hours after the preceding one.

Since the main difference between the anterior and posterior fragments consisted in the presence or absence of the ciliary apparatus, it would appear that the advance in onset of mitosis in the posterior halves was a consequence of the diversion of energy and precursors from processes concerned with the operation of the ciliary apparatus to mitosis. We suggest that the loss of the ciliary apparatus causes a considerable decrease in the utilization of adenosine triphosphate, and that this results in a reduced level of adenosine diphosphate available for further phosphorylation. An inadequate amount of adenosine diphosphate as phosphate acceptor would result in a shift toward a reduced state of the respiratory chain, as demonstrated in in vitro experiments by Chance (*6*) and by Lardy (*7*). An increase in both phosphorylation pressure and reducing power would indeed satisfy some of the known requirements for mitosis, such as the need for deoxyribose and triphosphates (*8*) as specific building blocks for the chromosomes and the need for a disulfide reductase activity (*9*) to initiate the spindle mechanism, as suggested by Mazia (*10*).

Weisz' observation (*11*) that the fusion of enucleated fragments of *S. coeruleus* with nucleated individuals in early interphase advanced the onset of mitosis considerably is also pertinent here, since Brachet has shown (*12*) that aerobic adenosine triphosphate production in enucleated cell fragments continues undiminished. The fusion of an enucleated fragment with a nucleated cell therefore brings about a relative increase in the production of chemically bound energy, while such energy-requiring processes as ribonucleic acid and protein synthesis (*13*), which are largely nucleus-dependent (*14*), probably do not show a proportional acceleration. Plesner's recent finding (*15*) that nucleoside triphosphates accumulate in synchronized cultures of *Tetrahymena* just prior to mitosis substantiates the above interpretation and suggests that an imbalance similar to that produced in the experiments with *Stentor* is responsible for the initiation of mitosis towards the end of interphase during normal growth.

The role that competition for a supply of energy for various cellular processes plays in the regulation of mitosis is now being subjected to further scrutiny in the myxomycete *Physarum polycephalum,* which provides synchronized mitoses (*16*) in a mass of tissue large enough for chemical analysis.

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Instructions for preparing reports. Begin the report with an abstract of from 45 to 55 words. The abstract should *not* repeat phrases employed in the title. It should work with the title to give the reader a summary of the results presented in the report proper.

Type manuscripts double-spaced and submit one ribbon copy and one carbon copy.

Limit the report proper to the equivalent of 1200 words. This space includes that occupied by illustrative material as well as by the references and notes.

Limit illustrative material to *one* 2-column figure (that is, a figure whose width equals two columns of text) or to *one* 2-column table or to *two* 1-column illustrations, which may consist of two figures or two tables or one of each.

For further details see "Suggestions to Contributors" [*Science* 125, 16 (1957)].

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28 January 1959

Pepsin-like Enzyme in Larvae of Stable Flies

Abstract. A pepsin-like enzyme has been found in larvae of the stable fly. The activity is detectable in homogenates of whole larvae and in homogenates of isolated midguts, the latter of which possess a *p*H environment low enough to support a peptic enzyme. The *p*H optimum of the activity was determined to be 2.4.

Prior to the work of Greenberg and Paretsky (*1*), it was assumed that the enzyme pepsin was absent from insects, and indeed, was restricted to vertebrates. However, these workers showed that a pepsin-like activity is present in all three larval stages of the house fly. Although Champlain and Fisk (*2*) were unable to detect pepsin in homogenates of whole, adult, blood-fed stable flies, we felt that the results of Greenberg and Paretsky warranted a closer investigation in other stages of the stable fly, and also in the expected site of protein digestion, the midgut. The present report (*3*) summarizes the results of a study of a pepsin-like enzyme in the stable fly, *Stomoxys calcitrans* (L.), and the house fly, *Musca domestica* (L.).

Fully mature, third instar larvae, reared on CSMA medium after the method of Champlain *et al.* (*4*) were used throughout. Homogenates of whole larvae (final concentration 10 per milliliter) or dissected midguts (final concentration 50 per milliliter) were prepared with a Teflon-Pyrex tissue grinder in glycine-NaOH buffer (*p*H 2.5) in the cold, and the cuticular debris was removed by centrifugation. Homogenates were stored frozen before use. Estimation of pepsin-like activity was made essentially by the method of Fisk and Shambaugh (*5*) by incubating 0.1 ml of homogenate with 0.9 ml of glycine-NaOH buffer (*p*H 2.5) and 0.25 ml of sulfanilic acid azoalbumin substrate (*6*) at 37°C for 2 hours. Analysis of the diazotized, colored amino acid or peptide fragments, released in direct proportion to enzyme activity (*5*), was accomplished with a Klett-Summerson photoelectric colorimeter with the 420-mμ filter. Controls consisted of reaction mixtures containing homogenate which had been placed in a boiling water bath for 5 minutes prior to assay, and also of reaction mixtures in which the homogenate was replaced by an equal volume of buffer to determine autolysis and residual color of the substrate. Although the investigation was primarily qualitative, the same wet weight of tissue, or number of midguts or whole larvae, was used for comparable assays.

Table 1 is a summary of the results, which confirm the presence of a pepsin-like enzyme in both whole larvae and midguts of *Musca domestica* and *Stomoxys calcitrans*. There is a small but consistent increase in Klett-Summerson colorimeter units in the unaltered mixtures compared with the values obtained for boiled homogenate mixtures or with the residual color of the substrate alone. Variations occurred from preparation to preparation. An additional series of experiments was carried out in which the Folin-Ciocalteau technique employed by Greenberg and Paretsky was followed. By this technique we showed that larvae of both species possessed nearly equal pepsin-like activities. A later comparison of the activities of homogenates from third instar larvae, pupae, and adult stable flies showed that the larvae had the most activity, followed closely by the pupae, but with the adults quite low.

To insure that the enzyme activity was not due to microorganisms, tests were repeated with axenic larvae (*7*). Essentially the same results were obtained (see Table 1). Sterility checks on representative samples of media and larvae showed them to be negative for bacteria, yeasts, and molds.

It was also necessary to determine whether the larval stable fly possessed an alimentary *p*H acid enough to support peptic activity. An experimental procedure was followed (*8*) in which young third instar larvae were reared on enriched artificial medium plus *p*H indicator dyes. The larvae fed for 2 days and were then dissected to determine *p*H. The *p*H of the medium itself was also noted.

The results of the *p*H tests of the digestive tract clearly indicated that the anterior portion of the midgut, and part of the mid portion of the midgut actually could provide a suitable *p*H environment for peptic activity. The following *p*H ranges were recorded: anterior midgut, 2.8 to 3.0; middle midgut, 2.8 to 4.8; posterior midgut, 6.8 to 7.9; hindgut, 7.9 to 9.6. These conditions prevailed although the food itself was above *p*H 6.8 before ingestion.

The *p*H optimum was determined for the pepsin-like activity by measuring both *p*H and increase in Klett reading after 2 hours' incubation of reaction mixtures buffered at 0.3 *p*H intervals between *p*H 1.0 to 3.1. The highest activity was found at *p*H 2.4, but the activity was only slightly less at *p*H 2.8. Activity was lowest below *p*H 1.3. These results are similar to those of Greenberg and Paretsky (*1*) who found the optimum to be *p*H 2.5 in both whole homogenates and dissected guts of the house fly.

At present it is not known whether the enzyme activity is similar to true gastric pepsin of higher animals or is a cathepsin acting at low *p*H. It is also unknown

Table 1. Results of tests confirming presence of pepsin-like activity in *Musca domestica* (L.) and showing similar activity in third instar larvae of *Stomoxys calcitrans* (L). Except as noted, activities are averages of seven replicates, while boiled homogenate or homogenateless mixtures represent two replicates.

Enzyme source	No. of whole insects or guts per replicate	Reading		Net increase due to active homogenate	Reading of homogenateless mixture
		Active mixture*	Boiled homogenate		
		Normal larvae			
Musca					
Whole larvae	1	44.3	41.5	2.8	
Midguts	5	37.2	20.9	16.3	
Stomoxys					
Whole larvae	1	43.9	36.5	7.4	21.0
Midguts	5	32.8	21.0	11.8	19.9
Midguts	5	23.7	13.4	10.3	8.5
Midguts	5	18.4	12.0	6.4	8.5
		Axenic larvae			
Stomoxys					
Whole larvae	4	16.5†	0‡	16.5	
Fore and Midguts	4	18.3†§	0	18.3	

* Klett units (Klett-Summerson photoelectric colorimeter with 420-mμ filter).
† Klett units derived from optical density readings with Spectronic 20 colorimeter at 440 mμ.
‡ Readings made with boiled homogenate tubes arbitrarily set at 0.
§ Activity readings here represent just six replicates.

whether the observed activity has a functional significance in the alimentary tracts of these insects. A series of tests with homogenates made from larvae from which the alimentary tracts had been removed were negative for pepsin-like activity, indicating that the gut tissue is the principal source of the enzyme.

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Biogenesis of Nicotine

Abstract. Radioactive nicotine was synthesized by *Nicotiana rustica L.* which was fed sodium acetate-1-C^{14}, sodium acetate-2-C^{14}, sodium pyruvate-1-C^{14}, or sodium pyruvate-3-C^{14}. Acetate-2-C^{14} and pyruvate-3-C^{14} were converted to nicotine with the least dilution of radioisotope, whereas pyruvate-1-C^{14} was incorporated with a relatively large dilution. When acetate-1-C^{14} was administered to the plants the nicotine contained C^{14} only in the pyrrolidine ring. After acetate-2-C^{14} was fed, C^{14} was located in both the pyrrolidine and pyridine rings.

Biogenetic pathways for the synthesis of the pyrrolidine and N-methyl groups of nicotine in tobacco plants have been firmly established (*1, 2*). Although Dawson *et al.* (*3*) have demonstrated the incorporation of the pyridine ring of nicotinic acid into nicotine, the *de novo* biogenesis of the pyridine ring of nicotine or other pyridine compounds in higher plants has not been elucidated. It has been shown that nicotinic acid is synthesized from tryptophan in several microorganisms and animals (*4*). However, higher plants do not appear to be capable of synthesizing the pyridine ring system of nicotine, trigonelline, or niacin from tryptophan (*5, 6*).

Recently, Il'in showed that acetate-C^{14} is incorporated to a large extent into nicotine in tobacco plants (*7*) but did not ascertain the location of the radioactivity in the nicotine molecule. Leete *et al.* (*8*) also demonstrated incorporation of acetate-2-C^{14} into nicotine and indicated that the radioactivity was randomly distributed in the alkaloid.

This report is a study of the incorporation of acetate-1-C^{14}, acetate-2-C^{14}, pyruvate-1-C^{14}, and pyruvate-3-C^{14} into nicotine in tobacco plants and presents data of a preliminary degradation to discover the location of radioactivity in nicotine following the feeding of radioactive acetate.

Intact *Nicotiana rustica L.* were prepared for hydroponic administration of the C^{14}-labeled substrates as described in an earlier report (*5*). Each plant was fed 2.44×10^{-5} mole of a given compound which had a specific activity of 4.10×10^8 count/min per millimole. The plants were harvested 7 days after administration of a labeled compound, and the nicotine was purified and isolated as the dipicrate (*9*).

Nicotine was degraded by oxidation with neutral potassium permanganate to yield nicotinic acid, potassium bicarbonate, and methylamine (*10*). In this procedure 3 moles of potassium bicarbonate were formed from the 3-, 4-, and 5-carbons of the pyrrolidine ring. The nicotinic acid was then decarboxylated, and the carboxyl carbon, which originally was the 2-carbon of the pyrrolidine ring of nicotine, was obtained as barium carbonate (*11*). The pyridine resulting from the decarboxylation was recovered as the picrate. The N-methyl group of nicotine was obtained by treatment of the alkaloid with hydriodic acid and conversion of the resulting methyl iodide to methyltriethylammonium iodide (*9*).

The radioactivity of each compound was determined with a windowless flow counter, and all counts were corrected for self-absorption.

The extent of incorporation of labeled substrates into nicotine is shown in Table 1. Acetate-2-C^{14} and pyruvate-3-C^{14} were converted to nicotine with the least dilution, whereas pyruvate-1-C^{14} was incorporated only to a relatively small extent. These results indicated that pyruvate was metabolized to acetate for the most part before it was incorporated into nicotine. However, the extent of conversion of pyruvate-3-C^{14} and the small but significant incorporation of C^{14} from pyruvate-1-C^{14} are an indication that not all pyruvate was converted to acetate before it was utilized for nicotine synthesis.

Nicotine which was synthesized in plants fed acetate-1-C^{14} and acetate-2-C^{14} was partially degraded to determine the location of C^{14} in the molecule in each case. The results of these degradations are presented in Table 2. When acetate-1-C^{14} was the labeled precursor, about one-half the activity was located in the 2-carbon of the pyrrolidine ring. The remaining C^{14} was located in the barium carbonate obtained from the 3-, 4-, and 5-carbons of the pyrrolidine ring. Previous studies, in which nicotine was degraded following administration of ornithine-2-C^{14} (*12*) and glutamate-2-C^{14} (*1*), have shown C^{14} in the alkaloid to be equally distributed between positions 2 and 5 of the pyrrolidine ring. The precursors of the pyrrolidine ring therefore appear to pass through a symmetrical intermediate during the synthesis of nicotine. Since acetate was probably incorporated first into glutamate by way of the tricarboxylic acid cycle, the quantity of the C^{14} in the 2- and 5-carbons of the pyrrolidine ring should be equal. In the present study one-half the C^{14} in nicotine obtained from plants fed acetate-1-C^{14} was found in position 2 of the pyrrolidine ring. It is postulated, therefore, that the remaining C^{14} resides in position 5 of the pyrrolidine ring. Essentially no C^{14} was incorporated into the pyridine ring when plants were fed acetate-1-C^{14}. In contrast, radioactive carbon in the nicotine from plants fed acetate-2-C^{14} was located in both rings. The pyrrolidine ring contained about 60 percent of the total C^{14} in the molecule. If one again assumes an equal distribution of radioactivity between carbons 2 and 5, these positions contained about 20 percent of

Table 1. Incorporation of several labeled precursors into nicotine in tobacco plants.

Substrate	Nicotine dipicrate (10^5 count/min mmole)	Dilution
Acetate-1-C^{14}	4.50	911
Acetate-1-C^{14}	4.18	981
Acetate-2-C^{14}	7.47	550
Acetate-2-C^{14}	12.70	323
Pyruvate-1-C^{14}	0.49	8370
Pyruvate-3-C^{14}	16.90	243

Table 2. Distribution of C^{14} in nicotine from plants fed sodium acetate-1-C^{14} and sodium acetate-2-C^{14}.

Compound	Specific activity (10^3 count/min mmole)	
	Acetate-1-C^{14}	Acetate-2-C^{14}
Nicotine dipicrate	9.3	21.0
Barium carbonate*	3.7	8.6
Nicotinic acid	4.5	9.3
Methyltriethylammonium iodide	0.1	0.6
Barium carbonate†	4.4	1.8
Pyridine picrate	0.4	8.6

* Barium carbonate from the 2-, 3-, and 4-carbons of the pyrrolidine ring. The specific activity was multiplied by 3.
† Barium carbonate resulting from the decarboxylation of nicotinic acid.

the radioactivity of the original nicotine molecule, and about 40 percent was located in carbons 3 and 4 of the pyrrolidine ring. Approximately 40 percent of the C^{14} in the nicotine was located in the pyridine ring.

Even though a 7-day period of metabolism was used with the radioactive acetate, only a small amount of randomization of the C^{14} in the synthesized nicotine occurred. These data also show for the first time the utilization of a specific precursor for the *de novo* synthesis of the pyridine ring in a higher plant (*13*).

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Nucleoprotein Constituents Stimulating Growth in Tissue Culture: Active Protein Fraction

Abstract. A new method has been developed for removing the nucleic acid portion of the nucleoprotein fraction which stimulates growth in tissue culture. Biological activity resides in the purified protein fraction, while the high-polymer nucleic acid fraction is inert. The active protein fraction contains extractable lipids which have no effect on the biological activity.

As was previously reported, the factors of high molecular weight in embryo extract which are needed for stimulation of certain types of tissue culture can be isolated with streptomycin as a protein precipitate both from embryo extract (*1*) and from adult tissues (*2*). These growth factors, which are active in both plasma and glass substrate tissue cultures (*3*), are obtained from the streptomycin precipitate in the form of a nucleoprotein fraction (NPF) (*1*). Since the nucleoprotein fraction is a mixture containing four electrophoretic components (*4*), further fractionation was attempted to identify the individual active growth principles.

In view of recent work on viruses (*5*) demonstrating biological activity in a specially prepared nucleic acid fraction of very high molecular weight, attempts were made to obtain similar nucleic acid fractions from the nucleoprotein fraction, although earlier, lower polymer nucleic acid preparations were inactive (*1*). Various new methods for isolating these high polymers (*6*) were tried with the nucleoprotein fraction and chick embryo homogenates; these resulted in nucleic acid preparations of varying polymer size, but all nucleic acid preparations were inactive in culture (*7*). Although most of the protein residues from these experiments were insoluble in salt solutions and inactive in cultures, some protein residues from these experiments partially redissolved and possessed growth-promoting activity when tested in tissue culture. This suggested the possibility of an active soluble protein fraction. Separation of soluble proteins from nucleic acids in the nucleoprotein fraction was attempted on the basis of an earlier observation (*7*) that a high concentration of phosphate buffer caused the nucleoprotein fraction to separate in this dense medium and to form a floating scum. Since high salt concentrations are frequently used to split off nucleic acids from proteins, the nucleic acid was presumably split off in this instance. More important, the floating material redissolved easily in buffers of low ionic strength (*7*).

On reinvestigation the flotate was indeed found to exhibit a typical protein absorption spectrum with a maximum of 278 mμ, in contrast to the spectrum of the nucleoprotein fraction, with a maximum of 260 mμ (*1*). This change suggested a loss of nucleic acid from the nucleoprotein fraction to form the protein fraction (PF); this was confirmed by chemical analysis for nucleic acid (*8*): NPF, 6.5 percent; PF, 0.8 percent (on a dry weight basis).

The growth-promoting activity of the protein fraction was tested on chick embryo heart fibroblasts in Carrel flask cultures, as previously described (*1*). The potency of the protein fraction was found to be unchanged from that of the nucleoprotein fraction, while the nucleic acid moiety was inactive (Table 1). The dose-response curves were alike, and similar to those published for the nucleoprotein fraction (*2*). Likewise, as already reported (*3*), no essential loss of biological activity was observed when a protein fraction was isolated from the nucleoprotein fraction of adult chicken spleen and tested on chick myoblasts in glass substrate cultures. The protein fraction from adult spleen appeared to be more active than the protein fraction from the embryo in this test system.

Table 1. Biological activity of protein fraction in plasma clot cultures of chick embryo heart fibroblasts.

Component	Amount (mg/ml)	Areal outgrowth at 7 days* (mm^2)
Serum control		8.0 ± 1.5
Embryo NPF	0.4	36.3 ± 6.1
Embryo PF	0.4	42.4 ± 5.4
Embryo nucleic acid	0.4	10.3 ± 3.3
Embryo delipidized PF	0.4	33.2 ± 4.7

* Averages, with standard error, for six flasks.

A routine procedure for isolation of protein fraction is as follows (all steps at 0°C): Clear fresh nucleoprotein fraction in Gey's solution is treated with 2 volumes of 3.3*M* potassium phosphate buffer at *p*H 7.8. The white turbid solution is allowed to stand for 1 hour, then is centrifuged for 20 minutes at 28,000*g* in the Servall centrifuge to concentrate the floating insoluble proteins as a flotate. The subnatant, containing nucleic acids and some protein, is discarded, and the flotate is further compacted by spinning for 30 minutes at 144,000*g* in the Spinco ultracentrifuge. The solid flotate is dialyzed for 48 hours against 2000 volumes of 0.1*N* sodium bicarbonate in which the protein fraction is stable. This step removes phosphate and dissolves much of the solid flotate. The contents of the dialysis sac are then spun for 1 hour at 144,000*g*, the floating lipid is removed with a capillary pipette, and the clear midfraction (in concentrations up to 20 mg/ml) is saved as the protein fraction.

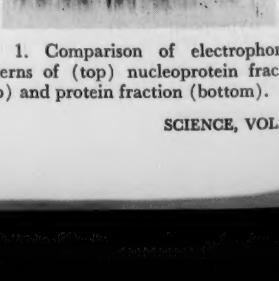

Fig. 1. Comparison of electrophoretic patterns of (top) nucleoprotein fraction (top) and protein fraction (bottom).

The bottom pellet is discarded. In contrast to the nucleoprotein fraction, the protein fraction can be sterile-filtered through ultrafine filters and frozen without loss of biological activity.

Since lipid components had been demonstrated in the nucleoprotein fraction (*9*), it was of some interest to determine whether they were still present in the protein fraction. Upon analysis (*10*), no significant change in total lipid content (dry-weight basis) was found in the protein fraction (4 percent) as compared to the nucleoprotein fraction (3 percent). By careful low-temperature extraction with acetone, followed by extraction with 2 : 1 chloroform methanol, it was possible to remove 90 percent of the total lipid present in the protein fraction. No significant change in biological activity was noted in the delipidized protein fraction (Table 1).

Ascending electrophoretic patterns of the embryo protein fraction 0.1*N* $NaHCO_3$ buffer, *p*H 8.8, were obtained (*11*) and are reproduced in Fig. 1, along with a typical ascending pattern of embryonic nucleoprotein fraction. The leading peaks have disappeared or have become greatly diminished, while the major peak remains intact. Similar patterns have also been obtained with this buffer at other *p*H values and with other buffers. It is therefore evident that the protein fraction is more electrophoretically homogeneous than the nucleoprotein fraction.

In view of these results, it appears that the biological activity is independent of the presence of at least 90 percent of the nucleic acid or lipid in the nucleoprotein fraction. Since the biological activity of the embryo nucleoprotein fraction can be completely accounted for by the delipidized protein fraction, it is planned to examine the constituents of the latter for the source of biological activity. First, the very small trace residues of nucleic acids and lipids still bound to the remaining proteins should be separated, either individually or as nucleolipoproteins, and tested. In view of the inertness of the nucleic acid and lipid fractions heretofore extracted and tested in culture (*1, 9*), it is not very likely that the biological activity resides here. Second, we must consider the possibility that tightly adsorbed or complexed large or small molecules, chemically dissimilar from the majority of the proteins in the protein fraction, are the active factors. Separation and biological testing of these tightly bound complexed molecules appears to offer a more profitable approach. Third, the electrophoretic pattern indicates a possibility of polydispersity of chemically similar proteins in the main component. Further separation and testing of polymers that differ in molecular size from the main electrophoretic component of the protein fraction may reveal a unique biologically active polymer. Finally, if all polymers in the main component are found to be equally active, separation, identification, and testing of disintegration products of the protein fraction may reveal its true nature. The method of large-scale continuous-flow paper electrophoresis alone, or in conjunction with chemical pretreatment, lends itself to evaluation of all of these possibilities (*12*).

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Nucleic Acids and Survival of Excised Anthers in vitro

Abstract. Excised anthers of *Allium cepa* and *Rhoeo discolor* have been successfully cultured in modified White's medium supplemented with various concentrations of ribonucleic acid and deoxyribonucleic acid. Ribonucleic acid proved to be much more useful than deoxyribonucleic acid and reduced the time required for the completion of meiosis from 48 hours to 24 hours. The role of nucleic acids in the control of nuclear divisions has been indicated.

In an earlier communication (*1*) I had reported the successful culture of the excised anthers of *Allium cepa* with the help of kinetin and gibberellic acid. Since then I have tried to rear anthers in improved culture media with the help of nucleic acids. A correlation between the development of microspore mother cells and the synthesis of nucleic acids in the anther has been shown by the work of several investigators (*2, 3*). In most plants the meiotic synthesis of deoxyribonucleic acid (DNA) is completed by the diplotene stage and anthers excised at or after diplotene give little difficulty in culture. On the other hand, all attempts to culture preleptotene anthers have been completely unsuccessful. Obviously, the most important steps in the differentiation of the microspore mother cells occur during the premeiotic interphase and perhaps in the early leptotene.

The importance of the tapetum in the development of pollen has often been emphasized, and Cooper (*4*) showed that in *Lilium henryi* and *L. regale* DNA is transferred from the tapetal cells to the microspore mother cells during meiosis. These observations have been regarded by some as fixation artifacts (*3*). However, Linskens (*5*) has found that in *L. henryi* after the beginning of prophase, DNA secreted by the degenerating tapetal cells and nuclei appears in the form of nucleotides and other degradation components, and is taken up by the reduced microspore mother cells for synthetic processes.

It is well established that the DNA content of the nuclei in the anthers increases at three specific stages during pollen development: (i) preleptotene, (ii) shortly before the division of the microspore nucleus, and (iii) shortly after the microspore mitosis, in the generative nucleus (*3*).

In the light of these observations I have used ribonucleic acid (RNA) and DNA (concentrations, 10, 25, 50, and 100 mg/lit.) for the culture of excised anthers of *Allium cepa* and *Rhoeo discolor*. White's modified medium was used for the experiments; the technique was the same as that reported earlier (*1*). Whole anthers were excised from the bud and cultured in Pyrex test tubes on an agar medium. For each test at least two dozen cultures (each with five anthers obtained from a single bud) were made with suitable control. The tests were usually replicated after about a fortnight.

The anthers of *Allium cepa* normally do not show any development in the basic medium. When the basic medium was supplemented with different concentrations of RNA (*6, 7*), the best development occurred at a concentration of 50 mg/lit. (Fig. 1). Anthers excised at leptotene-zygotene formed 68 percent dyads and 10 percent tetrads after 1 day along with 12 percent of the mother cells at leptotene-zygotene and 10 percent at diplotene-diakinesis; 16 percent dyads and 84 percent tetrads after 2 days; and 98 percent tetrads with 2 percent degenerated cells after 4 days. When excision was done at diplotene-diakinesis, there were 2 percent mother cells, 22 percent dyads, and 76 percent tetrads after 1 day; 6 percent dyads, 5

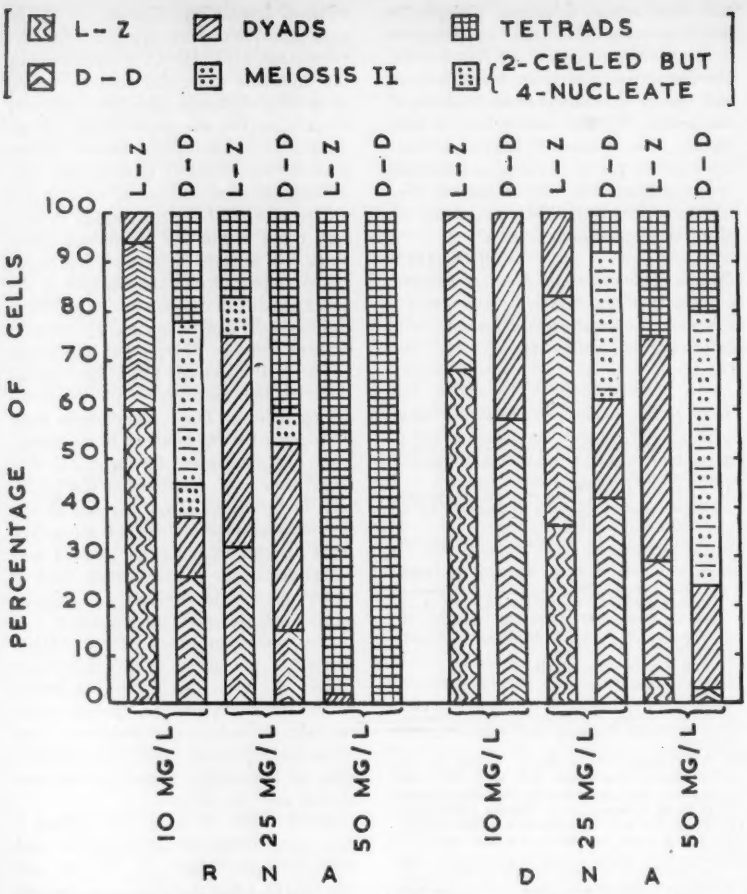

Fig. 1. Optimum development of anthers of *Allium cepa* excised at leptotene-zygotene (L-Z) and diplotene-diakinesis (D-D), in modified White's medium with different concentrations of RNA and DNA at 20°C.

percent mother cells in telophase II and 89 percent tetrads after 2 days; and 100 percent tetrads after 4 days (this is the best development obtained in *Allium cepa* anthers so far).

With DNA (*6, 7*) also, best results were obtained in *Allium cepa* at 50 mg/lit. (Fig. 1). Anthers excised at leptotene-zygotene showed 7 percent unchanged mother cells, 32 percent in diplotene-diakinesis, 39 percent at the dyad stage, and 22 percent at anaphase-telophase II after 1 day; 5 percent mother cells at leptotene-zygotene, 24 percent at diplotene-diakinesis, 46 percent dyads, and 25 percent tetrads after 2 days; thereafter there was no further development. When the anthers were excised at diplotene-diakinesis there were 3 percent mother cells at diplotene-diakinesis, 29 percent dyads, 58 percent at metaphase-anaphase-telophase of meiosis II, and 10 percent tetrads after 1 day; 3 percent unchanged mother cells, 21 percent dyads, 56 percent at metaphase-anaphase-telophase of meiosis II, and 20 percent tetrads after 2 days. No further development occurred.

In the basic medium the anthers of *Rhoeo discolor* showed best development only when they were excised at diplotene-diakinesis (57 percent undeveloped mother cells and 43 percent dyads; after this degeneration set in). Ribonucleic acid proved most favorable at a concentration of 25 mg/lit. Anthers excised at leptotene-zygotene showed 4 percent undeveloped mother cells, 16 percent at diplotene-diakinesis, and 80 percent dyads after 1 day; after 2 days there were 3 percent undeveloped mother cells, 76 percent dyads, and 21 percent tetrads. When anthers were excised at diplotene-diakinesis, all the mother cells formed tetrads within 1 day (*8*) and after 2 days one-celled microspores were formed.

In both the plants the optimum development obtained was up to the formation of one-celled microspores, and the division of the microspore nucleus could not be induced even when the anthers were excised at the tetrad or the one-celled microspore stage.

As is evident from the foregoing, whole anthers including the wall layers and the tapetum were cultured on the agar medium. Nevertheless, the extra nucleic acids supplied stimulated the development of the microspores a great deal. From this it would seem that the added nucleic acids are replacing some function normally provided by the rest of the plant, apart from the one provided by the tapetal cells.

Ribonucleic acid has been reported to increase growth and cell division in plant tissues (*7*). Hildebrandt *et al.* (*7*) have concluded that the growth of marigold and tobacco tissues was "strikingly stimulated by both filtered and autoclaved RNA at concentrations of 400 and 4000 mg per liter, while DNA had no effect at low concentrations and was strikingly inhibitory with 4000 mg per liter" (*9*). It may be pointed out here that the supply of RNA from the follicular cells (comparable to the tapetum) to the germ cells (comparable to the microspore mother cells) seems to be very widespread in animals, and it has been suggested that "a large amount of RNA is the cause, or at least one of the causes of the starting of meiosis" (*10, 11*).

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8. The time taken by *Rhoeo discolor* anthers, excised at diplotene-diakinesis, to form tetrads was reduced by 50 percent in vitro—that is, tetrads were formed within 24 hours, whereas in nature the time required is 48 hours.
9. In the present study also, RNA proved to be much more useful than DNA. Further, it seems likely, especially in the light of Linskens' observations, that hydrolyzed nucleic acids may have a greater influence on the growth of excised anthers. A detailed report is in press (*J. Exptl. Botany*).
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8 December 1958

Sex Peptide of Drosophila melanogaster

Abstract. All tested stocks of *Drosophila melanogaster* exhibit a peptide in males but not in females. The failure of some investigators to demonstrate its presence is attributable to their choice of chromatographic solvents. Acid hydrolysis confirms its peptide identity. Its presence in females transformed into males by the mutant *tra* implicates the sex-determining loci in its formation.

Chen (*1*) has recently reported failure to confirm our earlier reports of the chromatographic demonstration of a peptide among the free Ninhydrin-positive substances of adult *D. melanogaster* males and its absence in females (*2, 3*). In our work we had squashed whole flies directly on filter paper sheets and had performed two-dimensional, descending chromatography, using butanol : acetic acid : water as solvent in one dimension and phenol in the other. Chen, on the other hand, used extracts in 80 percent methanol and developed his chromatograms first with 70 percent *n*-propanol in the ascending direction and then with water-saturated phenol in the descending direction. The following simple experiment demonstrates that his failure to find the sex peptide is attributable to his choice of chromatographic solvents.

Four hundred males or females of an isogenic Oregon R stock were thoroughly ground in an all-glass homogenizer with 1.0 ml of each of the following solvents: 80 percent methanol, 80 percent ethanol, and water. The alcohol extracts were clarified by centrifugation. After initial centrifugation, the water extract was boiled for 2 minutes to denature proteins, and recentrifuged. Some (0.05 ml) of each extract was deposited on each of the chromatographic sheets (Whatman No. 1 filter paper, 46.5 by 57 cm), dried, and subjected to two-dimensional chromatography. Some sheets were developed according to the methods of Chen. Others were subjected to descending development in the first dimension (20 hours) with butanol : acetic acid : water (4 : 1 : 5 by volume), and in the second dimension (20 hours) with a solvent consisting of phenol (Merck) and 0.1*M* borate buffer (*p*H 8.3) in a ratio of 4 : 1 by volume. Ninhydrin-positive spots became visible on drying. Their identity had been established in previous work by means of cochromatography with pure amino acids (*4*).

Figure 1 depicts part of the results. In brief, the sex peptide is found in water, methanol, or ethanol extracts of males, but not in any of the extracts of females. When sheets are developed according to the methods of Chen, however, the peptide overlaps the spot produced by glutamine and could be overlooked even though its Ninhydrin color is somewhat more purple than that of glutamine. Its absence from the chromatograms of Kaplan *et al.* (*5*) may be explained in a similar fashion.

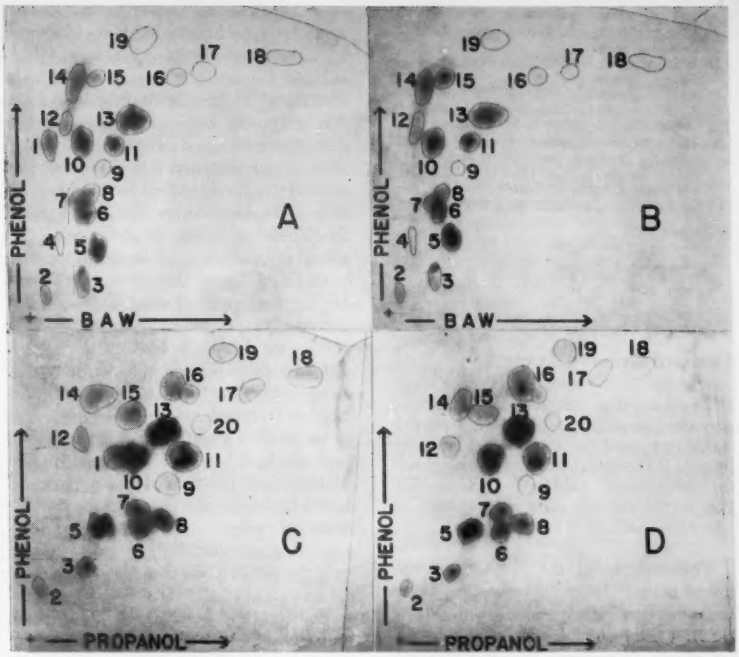

Fig. 1. Chromatographic separation of free Ninhydrin-positive substances of *D. melanogaster* extracted with 80 percent methanol. *A* and *C*, males. *B* and *D*, females. *A* and *B* were developed according to the method described in text; *C* and *D* were developed according to method of Chen (*1*). Identity of spots: 1, sex peptide; 2, peptide; 3, aspartic acid; 4, peptide; 5, glutamic acid; 6, serine; 7, taurine; 8, glycine; 9, threonine; 10, glutamine; 11, α-alanine; 12, lysine; 13, β-alanine; 14, arginine; 15, histidine; 16, methionine or methionine sulfoxide, or both; 17, valines; 18, leucines; 19, proline; 20, tyrosine.

When the appropriate portions of six or more sheets like that in Fig. 1*A* (not Ninhydrin-treated) are eluted with butanol : acetic acid : water (4 : 1 : 1) and the eluates are evaporated to dryness, sufficient sex peptide is obtained for further analysis. The products of hydrolysis of this material by concentrated HCl (110°C, 6 hours, followed by removal of HCl by evaporation) are water-soluble and yield four or five Ninhydrin-positive spots when subjected to chromatography. Our previous conclusion that this material is a peptide is thus confirmed.

We have now examined males of 16 different genotypes and females of 34 different genotypes, and in every case the sex peptide has been present in males and absent in females. This would indicate that this sexual difference is characteristic of the species as a whole, and it may also be true of other insects, particularly Diptera (*6*). We have previously demonstrated that this difference between males and females is attributable to the difference in number of X chromosomes (one in males, two in females) rather than to the presence and absence of the Y chromosome, thus suggesting the involvement of the sex-determining genes carried by the X chromosome (*3*). Examination (*7*) of the free Ninhydrin-positive substances of genetic females (2X/2A) transformed into sterile males by the third chromosome recessive mutant *transformer* (*tra, 8*) discloses that they possess the sex peptide. Since *tra* is a modifier of the genetic sex-determining system, the conclusion that the sex peptide is a product of that system is substantiated (*9, 10*).

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12 January 1959

Terminal Oxidase of Orchard Grass

Abstract. The response of infiltrated surviving green leaves to HCN, 1-phenyl-2-thiourea, and sodium diethyldithiocarbamate is consistent with a functional role for cytochrome oxidase in respiration. Polyphenol oxidase does not function as a terminal oxidase in orchard grass.

The identity of the functioning terminal oxidase in green leaves of grasses has not been clearly established. While studying losses of dry matter in the curing of hay, I therefore examined the terminal oxidase responsible for respiration in the leaves of green grass. Cytochrome oxidase has been reported in various plant tissues, but the pathway of electron transfer varies among different plants (*1*). Polyphenol oxidase has been regarded by many as a possible terminal oxidase, although there is little direct evidence of its primary function in undamaged cells (*2*). However, reports that it is at least partially functional continue to appear (*3*), so that it appears desirable to offer contrary evidence.

Daly *et al.* (*4*) reported that respiration of young leaves of barley was mediated by cytochrome oxidase. However, in older leaves respiration was not inhibited by carbon monoxide; thus the functional oxidase in mature leaves was left unknown. Deijs and his co-workers (*5*) showed that, as rye grass dried, its decline in respiration was paralleled by a similar decline in polyphenol oxidase activity. These workers attributed HCN inhibition of grass respiration to the effect of cyanide on polyphenol oxidase.

Orchard grass (var. Potomac) contains cytochrome oxidase and abundant amounts of polyphenol oxidase. Neither the intact leaf nor homogenates of it oxidizes ascorbic acid. Cytochrome oxidase appears to be the principal functioning terminal oxidase.

Grass of height 10 to 20 cm was homogenized for 40 seconds in a Waring blender with cold 0.1*M* phosphate (5 ml of buffer per gram of grass). For the assay of polyphenol oxidase the phosphate buffer was *p*H 6.5, for cytochrome oxidase, *p*H 6.8. Since addition of 0.2*M* sucrose and 0.001*M* ethylenediaminetetraacetate did not increase cytochrome oxidase activity, these compounds were usually omitted (*6*). For ascorbate oxidation, the homogenizing medium was 0.1*M* citrate-phosphate, *p*H 5.7 (*7*). Glass-distilled water was used throughout.

Cytochrome oxidase was manometrically measured in darkness at 30°C with 0.014*M* *p*-phenylenediamine as substrate. This enzyme is stimulated two- to threefold by the addition of $10^{-5}M$ exogenous cytochrome *c*. The most active fraction of cytochrome oxidase is sedimented in 20 minutes at 6230 *g* (average). For the assay of polyphenol oxidase, oxidation of catechol was followed manometrically (*8*). Polyphenol oxidase activity is not sedimented by 16,700 *g* in 20 minutes.

Several enzyme inhibitors were studied both in vitro and in surviving leaves. Grass was cut into pieces 1 to 2 cm long and vacuum infiltrated with inhibitors in 0.1*M* phosphate, plus 0.2*M* sucrose at *p*H 6.8, prior to respiration measurements. Table 1 shows that the effects of inhibitors in vivo are similar to those obtained with the cytochrome oxidase preparation but that they differ from those obtained with polyphenol oxidase. The stimulation of cytochrome oxidase and leaf respiration by diethyldithiocarbamate could be caused by its acting as a substrate for this oxidase (*9*). The response of intact green leaves to inhibitors is consistent with a functional role for cytochrome oxidase in respiration. Polyphenol oxidase cannot be the terminal oxidase in this issue.

Table 1. Effect of inhibitors on oxidases and on respiration of orchard grass.

Inhibitor	Percentage change in activity from control*		
	Polyphenol oxidase (in vitro)	Cytochrome oxidase (in vitro)	Infiltrated surviving leaves
$10^{-3}M$ HCN	− 90	− 100	− 40
1-phenyl-2-thiourea, saturated (*8*)	− 100	0	0
$10^{-3}M$ Na diethyldithiocarbamate	− 100	+ 16	+ 60

* The endogenous respiration of grass infiltrated with 0.1*M* phosphate, *p*H 6.8, averaged 270 μl of O_2 per hour per gram of fresh grass. Oxygen uptake per Warburg flask averaged 111 μl/hr for cytochrome oxidase, and 330 μl/hr for polyphenol oxidase.

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3 December 1958

Transmission of Rabies to Laboratory Animals by Bite of a Naturally Infected Bat

Abstract. An insectivorous bat that attacked a man in western Montana was induced to bite suckling mice. Subsequently the bat died, and brain and salivary gland suspensions were inoculated into other mice. Rabies virus was isolated from all three groups of mice.

In a review of the relationship between bats and rabies, Enright (*1*) noted that transmission of rabies by bite of insectivorous bats had not been demonstrated. Burns (*2*) failed to obtain transmission to monkeys, guinea pigs, and white mice by bites of experimentally infected *Tadarida mexicana* and *Antrozous pallidus*, although he found the saliva infectious by intracerebral inoculation. Stamm *et al.* (*3*) also found the saliva of one experimentally infected bat (*Myotis lucifugus*) infectious on intracerebral inoculation. However, what evidence exists for transmission of infection by the bites of insectivorous bats rests upon the occurrence of infection in human beings. At the present time, one well substantiated (*4*) and two possible (*5*) infections have been reported in North America. The present report records infection in white mice resulting from the bite of a naturally infected little brown bat.

The bat (*Myotis californicus californicus*) (*6*) was captured by elk hunters on 21 September 1958 in the Bitterroot Mountains of western Montana, where it attacked one of them twice. The first attack occurred at midday of a sunny day while the hunter was standing in camp. The bat suddenly appeared, lit on the front of his shirt, and bit the fabric. The hunter, a technician in the Rocky Mountain Laboratory, was aware

of the infectious potential of the animal and therefore did not touch it. In a moment the bat flew away and the men entered their cabin. When they emerged about 3 or 4 minutes later, the bat again lit on the shirt and again bit it. It was captured in a jar and brought to the laboratory the next day.

The bat was induced to bite one thigh of each of three 2-day-old mice. Its attack upon the mice was not exceptionally vicious. Three other suckling mice of the same litter were not submitted to bites but were kept in the litter as controls. This measure was found necessary because of occasional nonspecific mortality in litters. Neither the injected nor the normal mice were marked to distinguish them, since this might induce cannibalism in, or abandonment by, the mother.

The bat was kept in a glass jar with water and canned dog food available. Under these conditions, normal bats usually survive for a month or more but this one died 7 days after capture. The carcass was stored in Dry Ice until it could be examined.

On the 13th day after being bitten, two mice, and on the 14th day a third mouse, exhibited partial paralysis of the hindquarters. Their brains were removed for transmission. The remaining mice were observed for 28 days and remained normal throughout that period.

When it appeared that an infectious agent was present in the bitten mice, brain tissue and salivary glands of the bat were triturated separately in albumen-saline diluent and injected intracerebrally into 21-day-old white Swiss mice. Salivary-gland suspension was also injected intramuscularly. All injections were 0.02-ml volumes of 5- to 10-percent suspensions. On the 10th day afterward, four of six mice injected intracerebrally with brain suspension were obviously ill and were killed. Another mouse was sick on the 11th day, the sixth mouse on the 12th day; both were dead on the 14th day. None of the mice injected with salivary-gland suspension showed signs of illness during a 28-day period of observation.

An aliquot of the salivary-gland suspension not used in the first injections and which had been stored in a Dry Ice chest was then injected intracerebrally into six 21-day-old mice and into a litter of five 2-day-old mice. One of the litter became ill on the 14th day and was killed. Another, sick on the 21st day and held for observation, was dead the next day and was discarded. Three of the litter remained normal. None of the 21-day-old mice showed evidence of infection.

A transmissible agent was demonstrated in tissues of mice injected with tissues from, or bitten by, the bat. These tissues included brain of mice infected by biting or by injection of salivary glands and brain, and salivary glands of mice infected by biting. The isolates were neutralized by immune serum prepared in rabbits by hyperimmunization with PV-1 strain of rabies virus. Mice immunized with H.E.P. Flury strain virus (*7*) were immune to intracerebral injection of the bat isolates.

A hemisphere of brain from a mouse bitten by the bat was fixed and stained with William's stain. It contained numerous Negri bodies (*8*).

The occurrence of human rabies following bat bite and this demonstration of infection in bitten laboratory animals establish the infectiousness of bites of insectivorous species. While little doubt existed that such would be found eventually, the potential is now clearly demonstrated. The fact that three mice were bitten and three, presumably the same ones, succumbed at about the same time probably indicates that all were infected. Greater susceptibility of young mice (*9*) infected by any route may have, in part, contributed to the efficacy of transmission in this case. This certainly seemed to be true when a salivary gland suspension was injected into suckling and into weanling mice. Perhaps the thin skin of 2-day-old mice may also have facilitated transmission.

Because the bat died before the bitten mice became ill, a second test of the bite to determine persistence of infectiousness was not made. Unusual behavior in captivity was not noted, but opportunities for activity were limited in the close confines of the jar. It has yet to be demonstrated that insectivorous bats can become true carriers of infection—that is, infective but symptom-free. If such were the case, and if they lacked the aggressive urge of furious rabies, they, unlike the vampire bats, would not constitute a direct and serious menace to animals other than bats. However, the potential for spread within a colony as a result of intraspecific strife might be increased.

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28 January 1959

Identification and Assay of 5-Hydroxytryptamine in Molluscan Tissues by Fluorescence Method

Abstract. The fluorescence assay method has been used in the identification and quantitative estimation of 5-hydroxytryptamine (5-HT) in mollusks. Ganglia of a representative pelecypod contain more 5-HT than those of a gastropod. Most non-nerve tissues have low levels of 5-HT, which, except in "kidneys," may derive from nerve endings.

By means of paper chromatography and bioassay, 5-hydroxytryptamine (serotonin, enteramine) was found in nerve tissue of the mollusks *Venus mercenaria* and *Busycon canaliculatum* (*1*). This earlier identification has now been confirmed, non-nerve tissues have been examined, and the quantities of 5-HT have been more precisely determined, by the spectrofluorometric method developed at the National Institutes of Health (*2*). We have followed the analytical procedure outlined by Bogdanski *et al.* (*3*), with minor modifications, such as a lower ratio of tissue weight to solvent

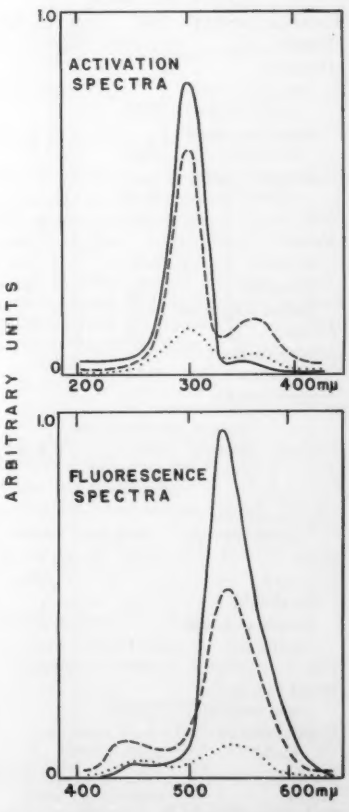

Fig. 1. Activation and fluorescence spectra of authentic 5-HT (solid line), extract of *Venus* ganglia (dashed line), and extract of *Busycon* ganglia (dotted line), all in 3*N* HCl.

volumes, made necessary by the small amounts of tissue available. With this method, we obtained activation and fluorescence spectra of extracts of nerve tissues and of authentic 5-HT in 3*N* HCl, such as are shown in Fig. 1. These are the observed curves; activation maxima appear at 305 mμ and fluorescence maxima at 540 mμ (*4*). In publications from the National Institutes of Health (*2, 3*), the activation maximum of 5-HT in 3*N* HCl is given as 295 mμ, that for fluorescence as 550 mμ. However, the maxima for the extracts and the authentic 5-HT, as is shown in Fig. 1, are in good agreement, and it is now reasonably certain that 5-HT has been correctly identified in tissues of mollusks. Dimethyl-5-hydroxytryptamine (bufotenin) has the same activation and fluorescence maxima as 5-HT, but it has not been seen on chromatograms of *Venus* or *Busycon* ganglia.

Table 1. 5-Hydroxytryptamine content of tissues of *Venus mercenaria* and *Busycon canaliculatum*.

Tissue	5-HT (μg/g)	Range
Venus mercenaria		
Ganglia (pooled)	30.0	12–52*
Blood†	< 0.004	
Digestive		
Intestine (visceral portion)	0.66	
Intestine (rectal portion)	0.60	
Digestive gland ("liver")	0.10	
Gill	0.53	
Heart		
Auricles	0.20	
Ventricle	0.50	
Bulbus arteriosus	0.04	
Kidney	0.26	
Mantle (central portion)	0.37	
Mantle (edge)	0.75	
Busycon canaliculatum		
Ganglia (pooled)	9.2	8.4–9.7‡
Nerve (connectives)	2.0, 2.5	
Ganglia and attached nerves	4.3, 5.5	
Blood	0.02	
Digestive		
Esophagus	0.06	
Intestine (rectal portion)	0.11	
Gill	0.23	
Heart (auricle and ventricle)	0.36	
Hypobranchial gland	0.08	
Kidney	2.0	1.4–2.3‡
Mantle	0.08	
Radula and odontophore muscles	0.07, 0.09	
Salivary glands	< 0.01	

* Eleven determinations. † With some mantle fluid. ‡ Four determinations.

Levels of 5-HT in various representative tissues of *Venus* and *Busycon* are given in Table 1. No correction has been made for the failure of the method to extract all the 5-HT, and with our modified procedure we recover about 70 percent of added 5-HT.

The *Venus* ganglia examined included the cerebropleural, visceral, and pedal. The *Busycon* ganglia examined included the entire esophageal complex but not the visceral ganglia. In two experiments, for which results are not shown, groups of similar ganglia of *Venus* were pooled and extracted separately. No consistent differences between the three groups were found. In each of the 11 separate determinations on *Venus* ganglia of Table 1, pooled tissues from two to ten animals were used. The rather large spread of values is due in part to seasonal and individual variation in 5-HT content and in part to the difficulty of freeing the small, fragile *Venus* ganglia of surrounding tissue. The mean value of 30 μg of 5-HT per gram of fresh tissue is much higher than has been found in nerve tissue of any vertebrate, and we have found equally high values only in ganglia of other species of pelecypod (bivalve) mollusks. Only in organs where 5-HT is a component of a venom are levels of 5-HT very much in excess of 30 μg/g (*5*). The 5-HT content of *Venus* tissues other than ganglia is low. The mantle edge, a well-innervated, muscular structure, has only 0.75 μg of 5-HT per gram, while a presumably poorly innervated organ such as the digestive gland has only 0.1 μg of 5-HT per gram. One determination on *Venus* blood failed to give a detectable amount of 5-HT.

Ganglia of *Busycon* were found to contain about one-third as much 5-HT as those of *Venus*. Ganglia of several other species of gastropod mollusks have less 5-HT than do those of most bivalves. From *Busycon* it is possible to obtain nerve connectives. These were found to contain considerably less 5-HT than equal weights of ganglion tissue. Most non-nerve tissues of *Busycon*, like those of *Venus*, yield relatively small amounts of 5-HT. Of the tissues examined, only salivary glands failed to yield a detectable amount. The *Busycon* kidney was found to contain more 5-HT than any other non-nerve tissue.

The relatively large amount of 5-HT found in *Busycon* kidneys made it of interest to determine whether kidney homogenates would decarboxylate added 5-hydroxytryptophan. The homogenates were not able to do so; this finding suggests that the *Busycon* kidney may be concentrating and excreting intact 5-HT, rather than synthesizing it for a local function. Excretory organs of several other invertebrate species have been found to have a high content of 5-HT, and in *Limulus*, the horseshoe crab, the coxal glands yield considerably more 5-HT than an equal weight of tissue of the central nervous system (*6*).

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6. The work reported here is a portion of a more extensive survey of the occurrence of 5-HT in invertebrates, especially in their nervous systems. The survey is supported by research grant B-623 from the National Institute of Neurological Diseases and Blindness, National Institutes of Health.

28 November 1958

Linear Titration Curves

In an interesting note in this journal (*1*), N. R. Joseph pointed to the advantage of bringing the sigmoid form of titration curves based on the mass action law into a linear form by logarithmic transformation. This transformation was applied by Joseph especially to the Henderson-Hasselbalch equation; he constructed a semilogarithmic paper as well as a slide rule for estimation of the *pK* values.

For such reasons some years ago I proposed a similar logarithmic transformation of the equation of the mass action law in its general form (*2*)

$$x^n K = y/(1-y)$$

yielding

$$n \log x - pK = \log [y/(1-y)]$$

At the same time the production of a corresponding (double) logarithmic paper for a linear representation of such titration curves was recommended, in which $\log [y/(1-y)]$ is plotted on the ordinate as a percentage of $y/(1-y)$ and $n \log x - pK$ is plotted on the abscissa. This paper is now available (*3*) and may be useful for special purposes.

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29 December 1958

Linear Titration Curves of Acids and Bases

Abstract. The Henderson-Hasselbalch equation, by a simple transformation, becomes $pH - pK = pA - pB$, where pA and pB are the negative logarithms of acid and base concentrations. Sigmoid titration curves then reduce to straight lines; titration curves of polyelectrolytes, to families of straight lines. The method is applied to the titration of the dipeptide glycyl aminotricarballylic acid, with four titrable groups. Results are expressed as Cartesian and d'Ocagne nomograms. The latter is of a general form applicable to polyelectrolytes of any degree of complexity.

By means of a simple transformation, titration curves of univalent weak acids and bases may be plotted as straight lines (*1*). The Henderson-Hasselbalch equation is converted to

$$pH - pK = pA - pB \qquad (1)$$

where pA and pB are negative logarithms of the acid and base concentrations. Plotted on semilogarithmic paper, the standard curve requires no reference to logarithmic tables. A special double logarithmic paper has been designed by Druckrey for general formulations of the mass action law (*2*). This may likewise be used to obtain linear graphs.

For polyvalent electrolytes, amino acids, peptides, or polypeptides, the titration may be represented by a family of parallel straight lines. The position of each line is determined by the value of pK for the group which is represented. The general method may be illustrated by its application to a dipeptide, glycyl aminotricarballylic acid (*3*). This contains four titrable groups: three carboxylic acid groups and the amino group. At any pH the equilibrium is represented by four reactions involving hydrogen ions:

$$A_1 = B_1 + H^+$$
$$A_2 = B_2 + H^+$$
$$A_3 = B_3 + H^+$$
$$A_4 = B_4 + H^+$$

The three undissociated carboxylic acid groups are denoted A_1, A_2, and A_3, while the respective ionized forms are denoted B_1, B_2, and B_3. A_4 and B_4 represent, respectively, the positively charged and neutral forms of the amino group. Corresponding to these four reactions are four transformed mass action law equations:

$$pH - pK_1 = pA_1 - pB_1$$
$$pH - pK_2 = pA_2 - pB_2$$
$$pH - pK_3 = pA_3 - pB_3$$
$$pH - pK_4 = pA_4 - pB_4$$

The equilibrium at any pH is determined by the pK values. These are, in order: 2.70, 4.10, 5.35, and 8.32 (*3*). The state of equilibrium over any range of pH is given by the family of four parallel straight lines (Fig. 1). These constitute a linear Cartesian nomogram. This form of diagram is much simpler than a family of four sigmoid curves requiring logarithmic calculations. In addition, the diagram clearly indicates the distribution of electrical charge over the molecule as a function of pH. The graphical method is applicable to polyelectrolytes of any degree of complexity. For a protein it would illustrate the distribution of electrical charges over all titrable groups in any given pH range—for example, in the neighborhood of the isoelectric point.

Cartesian nomograms consisting of families of parallel straight lines are readily transformed to d'Ocagne nomograms (*4*). The linear form of a simple titration curve (Eq. 1) is represented by three equally spaced parallel straight lines (Fig. 2). The first line is a scale of pH over any desired range. The second is the scale of $(pA - pB)$, or $\log B/A$. The third scale represents pK over the same range as that taken for pH. In the standard nomogram, values of pH and pK from 2 to 10 are represented. Each scale is linear, with pH and pK intervals equal. The pK scale is inverted with respect to the pH scale. The scale denoting $(pA - pB)$ is equidistant from the other two and parallel to them. It likewise is linear, with the mid-point at zero. Any value on this scale corresponds to the difference $(pH - pK)$ obtained by connecting with a straight line two points on the pH and pK scales.

The nomogram represents a generalized solution of Eq. 1. It can be used for univalent electrolytes, such as acetic acid, with only one pK value, or for polyelectrolytes of any degree of complexity. In Fig. 2, the dipeptide glycyl aminotricarballylic acid is represented. The four pK values (2.70, 4.10, 5.35, and 8.32) are indicated on the pK scale. The isoelectric point (3.41) is indicated on the pH scale. The state of ionization of each group is represented by the point of intersection with the middle scale of the line connecting pH with pK. The four connecting lines represent the distribution of charge over the four titrable groups as a function of pH. These relationships are not readily shown by the usual type of simple or compound sigmoid titration curves.

Especially in biological systems containing polyvalent titrable components is it advantageous to apply linear transformations of mass action law reactions. Redox potentials, usually described by sigmoid mass action formulations, may also be transformed to linear functions (*5*). When the redox potential depends on the pH as well as on the state of oxidation, the usual graphic formulation results in complex three-dimensional sigmoid surfaces (*6*). In biological preparations, complex polyelectrolyte systems

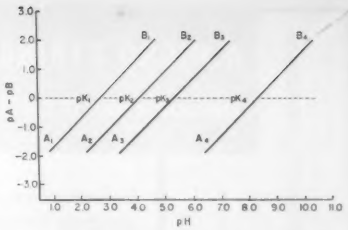

Fig. 1. Linear transformation of titration curve of glycyl aminotricarballylic acid (*3*), a Cartesian nomogram based on four pK values: 2.70, 4.10, 5.35, and 8.32.

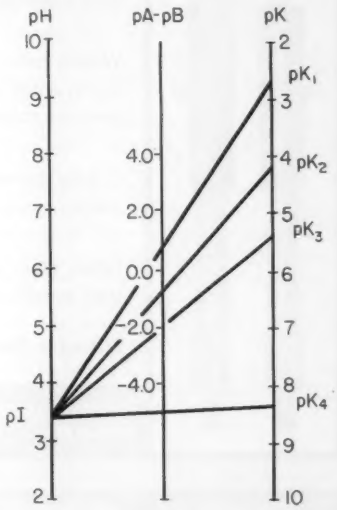

Fig. 2. Generalized d'Ocagne nomogram applicable to polyelectrolytes. Values of pK of four groups of glycyl aminotricarballylic acid are indicated on the pK scale. The value of the isoelectric point (pH 3.41) is shown on the pH scale. Simultaneous states of dissociation of the four titrable groups are indicated by the intercepts on the middle scale.

are often coexistent with one or more oxidation-reduction systems. By means of linear transformations of all the mass action formulations, physicochemical systems of high degrees of complexity may be depicted by simple types of linear nomograms.

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5 February 1959

Meetings

Science and Mathematics Teachers

The Central Association of Science and Mathematics Teachers (CASMT), a recent affiliate of the AAAS, was organized on 7 June 1902 as the Central Association of Physics Teachers. A committee from 25 schools met in Chicago on that date to consider organizing an association of physics teachers. At the meeting a constitution was adopted, and plans for a later meeting were developed. At the second meeting, held at the Armour Institute of Technology in Chicago, 9–11 Apr. 1903, the membership of the association was broadened to include teachers from all fields of science and mathematics. The larger organization was renamed the Central Association of Science and Mathematics Teachers. The aim of the association, ever since this meeting in 1903, has been to improve instruction in mathematics by introducing the laboratory method, and to bring about a closer correlation of mathematics with the various areas of the science curriculum, especially physics.

Beginning with the third meeting, conventions have been held annually during the Thanksgiving vacation period; meetings were held even during the war years. Ordinarily, meetings are held in Chicago for two consecutive years, then in one of the larger cities in the Great Lakes area. The attendance at such meetings varies from 500 to 1200. The membership, which now exceeds 1400, is not restricted to the Central States. Members come from all 49 states and from Canada, Europe, Asia, Africa, and Australia.

Communication among the members of the CASMT is maintained through the association journal, *School Science and Mathematics*. The journal features articles on research as well as the more scholarly variety of expository articles on course material and teaching in science and mathematics. The journal is now in its 57th year, and circulation exceeds 5000. Nine issues of the journal appear annually, from October through June. The present editor is George G. Mallinson.

The association, at recent meetings, has been emphasizing the relationships between science and mathematics and industrial processes and applications. Field trips through major industrial installations have been a special feature of the conventions and have been well attended. The CASMT, the only association that specifically emphasizes the relationships between science and mathematics, is undertaking a major examination of these relationships. Such activities are eminently important in view of the extensive reevaluation of both science and mathematics teaching at the present time.

The officers for 1958–59 are as follows: president, Clyde T. McCormick (Illinois State Normal University); vice president, F. Lynwood Wren (George Peabody College, Nashville, Tenn.); secretary, Joseph Kennedy (Indiana University); treasurer, Ray Soliday (Oak Park High School, Oak Park, Ill.).

GEORGE G. MALLINSON
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Film Congress in Britain

Representatives of documentary and scientific film organizations from many countries will meet at the 13th congress of the International Scientific Film Association, to be held in London and Oxford from 23 September to 2 October. This year it is expected that more than 200 films covering research, medicine, education, and popular science will be screened during the congress and its accompanying festival. The award-winning films will be shown at the National Film Theatre on 2 October. The British Scientific Film Association is organizing the conference. Further information may be obtained from the association's office at 3, Belgrave Sq., London S.W.1, England.

IAEA Nuclear Conferences

A symposium on radioactivation analysis will be held in Vienna, 1 to 3 June under the joint sponsorship of the International Atomic Energy Agency and the Joint Commission on Applied Radioactivity of the International Council of Scientific Unions. Other meetings on some practical aspect of the peaceful uses of nuclear energy have also been organized by the International Atomic Energy Agency.

In July, nearly 80 experts are expected to attend a seminar at Saclay, France, on the training of specialists in the peaceful uses of atomic energy.

A 6-day conference in Warsaw, Poland, on the application of large radiation sources in industry, especially to chemical processes, is scheduled for 5–11 September. Some 300 delegates are expected to participate.

Standardization of radioisotopes will be discussed at a symposium on radioactive metrology that is to be held in Vienna in October. About 100 participants, chiefly from national laboratories, will exchange views aimed at establishing internationally accepted methods of standardization.

The last in the series of meetings will be a conference on the disposal of radioactive waste, to be held in Monaco in

November. It will be designed to bring together scientists from major atomic energy establishments, as well as oceanographers, geologists, and other experts in the associated sciences. Papers will be presented and, simultaneously, there will be panel discussions on the roles oceanography and geology could play in dealing with the problem. An IAEA panel set up last October is studying the question of disposal in the sea, but in view of the complexity of the subject it was felt that a conference would supplement the panel's efforts.

Forthcoming Events

June

29–1. Military Electronics, 3rd natl. conv., Washington, D.C. (L. R. Everingham, Radiation, Inc., Orlando, Fla.)

29–3. Dairy Cong., 15th intern., London, England. (R. E. Hodgson, Animal Husbandry Research Div. Agricultural Research Service, U.S. Dept. of Agriculture, Washington 25.)

29–3. Problems in Pastoral Psychology (Inst. for the Clergy of All Faiths), New York, N.Y. (A. A. Schneiders, Committee for the Inst. for the Clergy, Dept. of Psychology, Fordham Univ., New York 58.)

29–3. Superconductivity, IUPAP colloquium, Cambridge, England. (D. Schoenberg, Dept. of Physics, Univ. of Cambridge, Mond Laboratory, Cambridge.)

29–4. Glass, 5th intern. cong., Munich, Germany. (P. Gilard, International Commission on Glass, 24, rue Dourlet, Charleroi, Belgium.)

30–10. International Electrotechnical Commission, Madrid, Spain. (IEC, 1–3, rue de Varembe, Geneva, Switzerland.)

July

1–3. Hydraulics, annual conf., Fort Collins, Colo. (W. H. Wisely, American Soc. of Civil Engineers, 33 W. 39 St., New York 18.)

1–4. British Tuberculosis Assoc., annual (closed), Cambridge, England. (BTA, 59, Portland Pl., London, W.1, England.)

1–5. International Radio and Electronics Conv., Cambridge, England. (British Institution of Radio Engineers, 9, Bedford Sq., London, W.C.1, England.)

2. Radiation and Ageing, Ciba Foundation 3rd annual lecture on ageing, London, England. (G. E. W. Wolstenholme, Ciba Foundation, 41 Portland Pl., London, W.1, England.)

3–5. International Union of the Medical Press, 4th cong., Cologne, Germany. (Dr. Stockhausen, Secretary of Bundesaerztekammer, Cologne.)

4–9. American Soc. of X-ray Technicians, Denver, Colo. (Miss G. J. Eilert, 16 14 St., Fond du Lac, Wis.)

6. Shortening of Lifespan of Mammals Following Irradiation, research forum, London, England. (G. E. W. Wolstenholme, Ciba Foundation, 41 Portland Pl., London, W.1, England.)

6–8. Cell Structure and Function, 10th annual symp., Ann Arbor, Mich. (J. M. Allen, Dept. of Zoology, Univ. of Michigan, Ann Arbor.)

6–8. Oxford Ophthalmological Cong., Oxford, England. (I. Fraser, 21, Degpole, Shrewsbury, Shropshire, England.)

6–8. School and University Health, 3rd intern. cong., Paris, France. (Comité d'Organisation du Congres d'Hygiene Scolaire et Universitaire, 13, rue du Four, Paris 6e.)

6–11. Seed Testing, intern. conv., Oslo, Norway. (Intern. Seed Testing Association, Danish State Seed Testing Station, Thorvaldsensvej, 57, Copenhagen V, Denmark.)

6–12. Chagas' Disease, intern. cong., Rio de Janeiro, Brazil. (C. Chagas, Instituto de Biofisica, avenida Pasteur 458, Rio de Janeiro.)

7–10. Royal Medico-Psychological Assoc., annual meeting, Glasgow, Scotland. (RM-PA, 11, Chandos Street, London, W.1, England.)

12–17. American Waterworks Assoc., annual conv., San Francisco, Calif. (H. E. Jordan, AWA, 521 Fifth Ave., New York 17.)

13–17. National Assoc. of Power Engineers, natl. conv., Boston, Mass. (A. F. Thompson, Secretary, NAPE, 176 W. Adams St., Chicago, Ill.)

13–17. Standardization, intern. (council meeting), Geneva, Switzerland. (ISO, 1–3, rue Varembe, Geneva.)

(See issue of 15 May for comprehensive list)

Letters

Francisco Duran-Reynals

The genuinely admirable qualities that characterized the career and personality of the late Francisco Duran-Reynals have been captured adroitly in the beautifully drawn portrait by C. C. Little [*Science* **129**, 881 (1959)]. I should like to add still another word in appreciation of Francisco Duran-Reynals, focusing upon a particular aspect of his life.

Francisco loved young people. Many of us came to know this as Jackson Laboratory summer students—even in fields far removed from microbiology. It was a common practice for scientists associated with the laboratory to give talks to the students, and Francisco's lectures were of particularly high quality. Informative and wonderfully lucid, they had an incisive logic and stylistic beauty that made us strain to hear every word.

It seems that he gave to these talks the same type of weighty consideration that would mark preparation of an address to a body of distinguished scholars. One could infer this, of course, from listening to him, but we acquired other evidence. For example, he and his lovely wife frequently invited us to "painting parties" at their house overlooking Bar Harbor. One Sunday afternoon when we came to fetch him, he was in his study and did not want to be disturbed. He was to talk to us the next morning, and we learned that he was terribly worried, lest it not be a success. He had been brooding over the lecture material all day.

When he emerged much later in the afternoon, I remember him taking me aside and commenting with warmth about the beauty of *l'heure exquise*—the hour before sundown which he loved so well. And, knowing of my own love for sunsets, he suggested I not despair at my impending return to New York City: Manhattan had sunsets, too; it was just that sometimes the tall buildings get in the way.

This consideration for us and our feelings became apparent in other ways, too. On the occasion of an important meeting in New York, some of us came to hear him, and lingering on somewhat gingerly at the end of the session, we wondered whether he would remember us and debated whether or not to greet him. But he spied us. Hurriedly excusing himself from eminent colleagues who had surrounded him, he rushed up the aisle, threw his arms around us, and, launching into exuberant conversation, shepherded us out of the hall. (How many of us today give so much *disinterested* attention?)

I do not know the extent of his lecturing at Yale. I do know that Francisco Duran-Reynals was a great teacher and a wonderful human being.

MICHAEL KAPLAN

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Balance in Cultivated Ecosystems

In their very interesting article on "Acrolein for the control of water weeds and disease-carrying water snails" [*Science* **129**, 335 (1959)], Overbeek *et al.* describe their experiments in ridding irrigation ditches of water weeds and fresh-water snails by the addition of small amounts of acrolein to the irrigation water. They state, "Treated water, when used for irrigation, did not harm crops. Further studies, on possible acrolein residues in crops and on the toxicity of treated water with respect to farm animals, are being made."

It is reassuring to know that attention is being given to possible toxicity to human beings and farm animals. It would be interesting to know, further, if any studies are contemplated on the effect of this treated water on the soil microbiota in the land that is being irrigated. Soil scientists reiterate constantly the importance of the soil fauna and flora in the development and maintenance in good condition of soils. It would be disastrous indeed to discover, after a few years of ditch-cleaning with this highly toxic substance, that the essential soil biota had been destroyed and that permanent or long-term impairment of large areas of irrigated soils had taken place.

Ecologists interested in the maintenance of a healthy balance in cultivated ecosystems should be much concerned about matters of this sort.

F. R. FOSBERG

Falls Church, Virginia

Fosberg will be glad to learn that in addition to tests of the effect of treated irrigation water on crops, soil microbiological tests have been made and are continuing.

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J. VAN OVERBEEK

Shell Development Company, Agricultural Research Division, Modesto, California

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